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Research Article

Investigation of the probiotic properties and acid production rate of *Lactobacillus delbrueckii ssp. bulgaricus*

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Abstract

Some of the basic requirements that lactic acid bacteria must meet before they are called probiotic are: marked antimicrobial activity, antibiotic susceptibility specific for the strain, being suited to industrial cultivation, ability to thrive in the food matrix, ability to maintain a high concentration of active cells in finished food products during both preparation and storage thereof and the ability to tolerate a low pH. The objective of the present study was to investigate the antimicrobial activity and acid-forming ability of selected strains *Lactobacillus delbrueckii ssp. bulgaricus* in order to determine which strain might augment the efficiency of starter cultures used in dairy production. The antimicrobial activity of *Lactobacillus delbrueckii ssp. bulgaricus* (four strains MG1, MG2, MG3 and MG4) against selected pathogenic microorganisms was investigated by the agar well diffusion method. The biomass, cultural medium and cell-free supernatant were tested, and the antibiotic susceptibility of the strains against antibiotics with different mechanisms of action was determined. The maximum specific growth rate and the parameters of the respective models for the selected strains were also determined. *Lactobacillus delbrueckii ssp. bulgaricus* MG3 had the highest antimicrobial activity along with susceptibility to a greater number of antibiotics. As a result of this study and mathematical modeling, we concluded that the MG3 strain is the recommended strain to increase the efficiency of starter cultures used in dairy production. Practical applications: This study was prompted by the need to produce starter cultures with established purity and activity for use in the food industry. The aim of this work was to investigate a specific group of lactic acid bacteria that are widely used in the composition of starter cultures.

Keywords: antimicrobial activity, antibiotic susceptibility, mathematical modeling, kinetics.

Abbreviations:

B – biomass

CM - cultural medium

BLIS - Bacteriocin-Like Inhibitory Substances

NCS - neutralized cell-free supernatant

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Introduction

Lactic acid bacteria help to maintain the balance of individual microbial species in the gastrointestinal tract through the production of short-chain acids (lactic, acetic, etc.), bacteriocins (Bhatia et al. 1989), BLIS (Bacteriocin-Like Inhibitory Substances) and other metabolites by suppressing enteropathogens (Davidson et al. 2000; Fang et al. 2001; Salminen and Wright 1998b; Salminen et al. 1999). The consumption of products containing lactic acid bacteria helps to protect the digestive system from disorders associated with imbalances in gastrointestinal micro flora (O'Brien et al. 1999). The probiotic properties of many lactic acid bacteria that have been incorporated into starter cultures for the production of various products and other fermented foods have been confirmed in recent years (Zhang et al. 2016; Puniya et al. 2016). Some of the basic requirements that lactic acid bacteria must meet before they are called probiotic are: marked antimicrobial activity; antibiotic resistance specific for the strain; being suited to industrial cultivation; ability to thrive in the food matrix; ability to maintain a high concentration of active cells in finished food products during both preparation and storage thereof, and the ability to tolerate a low pH (Padmavathi et al. 2018). This has led to the idea to combine mathematical and microbiological methods to further determine the properties of newly isolated strains *Lactobacillus delbrueckii ssp. bulgaricus*. Having reviewed the cited literature, the aim of the present study was to investigate the antimicrobial activity and acid-forming ability of the strains *Lactobacillus delbrueckii ssp. bulgaricus* in order to determine which strain might increase the efficiency of starter cultures used in dairy production.

Materials and Methods

Microorganisms

1. Four strains of lactic acid bacteria of the species *Lactobacillus delbrueckii ssp. bulgaricus* were isolated and identified from naturally fermented lactic acid products (yoghurt, cheese, etc.):

Lactobacillus delbrueckii ssp. bulgaricus MG1; *Lactobacillus delbrueckii ssp. bulgaricus* MG2; *Lactobacillus delbrueckii ssp. bulgaricus* MG3 and *Lactobacillus delbrueckii ssp. bulgaricus* MG4. The selected strains are property of the of "LB-Lact" laboratory in Plovdiv, Bulgaria.

2. *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 8739, *Listeria monocytogenes* and *Salmonella sp.* were used as test microorganisms.

Cultural medium. (Teneva et al. 2016a; Teneva et al. 2016b)

1. LBG - agar;
2. MRS (deMan, Rogosa and Sharpe) - agar;
3. MRS (deMan, Rogosa and Sharpe) - broth.

Determination of antimicrobial activity by the agar well diffusion method. The antimicrobial activity of the test strains was determined by means of the cultural medium (CM), biomass (B) and neutralized cell-free supernatant (NCS) (pH 6.5), obtained from a 24-hour culture of the respective strains. The antimicrobial activity was investigated against the following micro-organisms: *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 25923, and *Listeria monocytogenes*, *Salmonella sp.* (clinical isolate). Each of the test microorganisms (107 cfu/cm³) was inoculated into a Petri dish of agar medium and, after hardening of the agar wells, was prepared (7 mm). Next, 0.1 cm³ of CM, B was introduced into the wells and NCS and was allowed to stand for 1 h at 4° C and diffuse into the agar. The microorganism test plates were incubated at 37° C for 24 hours after which the inhibition zones were read in mm.

Determination of antibiotic sensitivity. A profile of antibiotic sensitivity was determined by the disk diffusion method of Bauer (Kirby et al. 1966). A fresh 24-hour culture of the strain was used to inoculate the MRS-agar plates. Standard discs impregnated with antibiotics were placed in the plates. The plates were then incubated for 48h at

44°C. The diameter (in mm) of the sterile zones formed around each of the antibiotic disks was recorded, and the following indications were used: R - resistant (zone <8 mm), SR - susceptible (zone 8 - 16 mm), S - sensitive (zone > 16 mm).

Cultivation of the strains tested. Cultivation of the tested strains was carried out under static conditions in sterile, reconstituted (12% dry matter) dry skim milk at $44 \pm 1^\circ\text{C}$.

Determination of the number of active viable cells. The number of viable cells was determined by the tenfold dilution method according to ISO 7889: 2005.

Determination of titratable acidity. Titratable acidity was determined according to the requirements of BDS 1111: 1980.

Modeling the kinetics of the fermentation process. To model the kinetics of the fermentation process, models 1 through 3 were used, as well as the Weibull model (Tishin and Fedorov 2016; Tishin and Golovinskaya 2015; Bouguettoucha et al. 2011; Gordeev et al. 2017). Identification of the model parameters was performed by minimizing the error between the experimental and calculated model data by the method of least squares and using the fourth line of the algorithm of Runge-Kutta (Kostov 2015).

Results and Discussion

The study of the antibiotic resistance of lactobacilli with pronounced probiotic properties is of special importance. For example, the antibiotic resistance of lactic acid bacteria is the basis of the selection of strains that are used in probiotics and in combined therapy with antibiotics to maintain and restore beneficial gastrointestinal micro flora [Charteris et al, 1998]. When studying the selection of antibiotic-resistant lactobacilli, it is worth noting the hypothesis voiced by a number of authors according to which lactobacilli may serve as a source of genes for antibiotic resistance by transferring the genes to different pathogens (Mathur & Singh, 2005). Consequently, in the selection of strains with proven probiotic potential, it is paramount to know the extent of their antibiotic susceptibility. To achieve this goal, 17 antibiotics divided in three groups,

based on their different mechanism of action were selected. The sensitivity of the newly isolated strains of *Lactobacillus bulgaricus* was then tested against these antibiotics. The results obtained by Bauer's agar diffusion method (Kirby et al. 1966) for 24 hours are summarized in Table 1. From the data presented in Table 1, it is evident that the newly isolated strains of *Lactobacillus bulgaricus* exhibit different sensitivity to the selected group of antibiotics. The MG1 strain shows a more pronounced resistance to antibiotics that inhibit cell wall synthesis compared to the other strains. Strain MG1 was resistant to ampicillin and vancomycin and susceptible to oxacillin. This strain exhibited sensitivity to amoxicillin + clavulanic acid and penicillin. Strain MG2 also showed susceptibility to oxacillin. The tested strain was susceptible to ampicillin and sensitive to vancomycin, penicillin and amoxicillin + clavulanic acid. The MG3 strain showed complete sensitivity to antibiotics that suppress the cell wall synthesis and the MG4 strain was also susceptible to this type of antibiotic. Compared to antibiotics that inhibit protein synthesis, the MG1 strain exhibited resistance to tetracycline and chloramphenicol and was susceptible to gentamicin, tobramycin and amikacin. The strain showed sensitivity to doxacycline, lincomycin and erythromycin. The MG2 strain was sensitive to almost the entire group of antibiotics inhibiting protein synthesis, was susceptible only to tobramycin and was sensitive to all other members of the group. For strain MG3, susceptibility to a wide range of antibiotics that inhibit protein synthesis was observed. For example, susceptibility to doxycycline, amikacin and tobramycin was detected. The test strain was also sensitive to tetracycline, gentamicin, lincomycin, chloramphenicol and erythromycin. The MG4 strain exhibited susceptibility to amikacin, gentamicin, tobramycin and erythromycin. *Lactobacillus bulgaricus* MG4 demonstrated sensitivity to tetracycline, doxacycline, lencomycin and chloramphenicol. The MG3 strain was susceptible to nalidixic acid and ciprofloxacin, and showed sensitivity to rifampin and clarithromycin. The MG4 strain was resistant to rifampin and was susceptible to nalidixic acid, ciprofloxacin and clarithromycin.

Table 1. Antibiotic sensitivity of *Lactobacillus bulgaricus* MG1, MG2, MG3, MG4

Mechanism of action of the antibiotics on bacteria	Antibiotic name and abbreviation		Concentration of the antibiotic	<i>Lactobacillus bulgaricus</i>			
				MG1	MG2	MG3	MG4
Inhibitor of cell wall synthesis	Penicillin	P	1 IU/IE/UI/disc	S	S	S	SR
	Ampicillin	A	10 µg/disc	R	SR	S	SR
	Oxacillin	O	1 µg/disc	SR	SR	S	SR
	Amoxicillin+ Clavulanic acid	V+CA	20/10 µg/disc	S	S	S	SR
	Vancomycin	V	5 µg/disc	R	S	S	SR
	Tetracycline	T	30 µg/disc	R	S	S	S
Protein synthesis inhibitors	Doxycycline	D	30 µg/disc	S	S	SR	S
	Gentamicin	G	10 µg/disc	SR	S	S	SR
	Tobramycin	Tb	10 µg/disc	SR	SR	SR	SR
	Amikacin	Am	30 µg/disc	SR	S	SR	SR
	Lincomycin	L	15 µg/disc	S	S	S	S
	Chloramphenicol	C	30 µg/disc	R	S	S	S
	Erythromycin	E	15 µg/disc	S	S	S	SR
	Rifampin	R	5 µg/disc	S	S	S	R
Inhibitor of DNA synthesis	Nalidixic acid	Nx	30 µg/disc	R	S	SR	SR
	Ciprofloxacin	Cp	5 µg/disc	SR	S	SR	SR
	Clarithromycin	Cl	15 µg/disc	S	S	S	SR

Legend: R-resistant, SR - susceptible (zone 7-16 mm), S - sensitive (zone> 16 mm)

Table 2. Antimicrobial activity of *Lactobacillus bulgaricus* MG1, MG2, MG3, MG4

<i>Lactobacillus delbrueckii ssp. bulgaricus</i> Strain	<i>Staphylococcus aureus</i> ATCC 25923	<i>Escherichia coli</i> ATCC 8739	<i>Listeria monocytogenes</i>	<i>Salmonella sp.</i>
Zone of inhibition (mm)				
MG1				
Biomass (B)	8	9	8	8
Cultural medium (CM)	8	8	8	8
Neutralized cell-free supernatant (NCS)	-	-	-	-
MG2				
Biomass (B)	9	10	10,5	10
Cultural medium (CM)	8	9	9	8
Neutralized cell-free supernatant (NCS)	8	9	9	-
MG3				
Biomass (B)	8	10	10	12
Cultural medium (CM)	8	9	9	9
Neutralizes cell-free supernatant (NCS)	-	8	8	9
MG4				
Biomass (B)	8	8	10	9
Cultural medium (CM)	8,5	8	9	8,5
Neutralized cell-free supernatant (NCS)	-	-	8	-

For strain MG2, sensitivity to all members of the group inhibitors of DNA synthesis was detected. MG1 showed resistance to nalidixic acid, was susceptible to ciprofloxacin and was sensitive to rifampin and clarithromycin. Strain MG1 exhibited resistance against a larger number of antibiotics compared to the other strains. Antibiotic resistance should be taken into account in the selection of probiotic strains due to their proven ability to transmit genes responsible for the emergence of resistance in pathogenic microorganisms (Mathur and Singh 2005). Therefore, with regard to the use of probiotics, together with antibiotics, the researchers would recommend not to intentionally select probiotic strains with a wider spectrum of resistance than what occurs naturally (Mathur and Singh 2005). For that reason, the strains should be screened for potentially transmissible resistance to chloramphenicol, clindamycin, gentamicin, rifampin and tetracycline (Danielsen and Wind 2003). One of the main criteria for the selection of probiotic microorganisms is their ability to enhance the host's natural protection against enteropathogens by producing antimicrobial substances or by competitively inhibiting and excluding these pathogens. (Morelli 2000). Consequently, studies have been conducted to determine the antimicrobial activity of newly isolated strains of *Lactobacillus bulgaricus* by the agar well diffusion method. For this purpose, a cultural medium (CM), biomass (B), and neutralized cell free supernatant were used. The results of this research are summarized in Table 2. The antimicrobial activity of the investigated strains was detected against the test microorganisms used. Strains MG2 and MG3 exhibited the most pronounced antimicrobial activity against the test micro-organisms compared to the other tested strains. Strains MG2 and MG3 exhibited similar antimicrobial activity. The antimicrobial activity and growth inhibition of *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 8739, *Listeria monocytogenes*, *Salmonella sp.* biomass and the cultural medium was observed for strain MG2. For this strain, the neutralized supernatant showed no antimicrobial activity on *Salmonella sp.* MG3 exhibited a similar spectrum of antimicrobial action but did not show antimicrobial activity against *Staphylococcus aureus* ATCC 25923 in the

neutralized supernatant. Strain MG1 showed antimicrobial activity against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 8739, *Listeria monocytogenes* and *Salmonella sp.* for biomass and the cultural medium. The cell-free supernatant did not exhibit a pathogen-inhibiting effect, suggesting the absence of bacteriocin-like substances. MG4 demonstrated activity against the tested pathogens for biomass and the cultural medium. However, the neutralized supernatant of this strain was only active against *Listeria monocytogenes*. From the results of the present study it can be concluded that of the tested strains, MG2 and MG3, demonstrated the most pronounced antimicrobial activity. These strains maintained antimicrobial activity in the neutralized cell-free supernatants against most of the pathogens. This outcome would suggest that the strains' high antimicrobial activity is likely due to the lactic acid produced and to the higher levels of diacetyl and bacteriocin-like substances with antimicrobial activity. The loss of antimicrobial activity of the neutralized cell-free supernatant against the test microorganisms for MG1 indicates that the antimicrobial activity of this strain is primarily influenced by the lactic acid accumulated in the medium. It does not imply the formation of bacteriocin-like substances. The cell-free supernatant of strain MG4 retained a certain level of antimicrobial activity against *Staphylococcus aureus* ATCC 25923. It can thus be assumed that for this strain, the antimicrobial activity was due to both lactic acid and other antimicrobial metabolites such as diacetyl and bacteriocin-like substances. The latter are apparently sensitive to the change in pH values, resulting in a change in the activity of these substances. A key requirement for the implementation of strains with pronounced probiotic properties for the production of probiotics and probiotic foods is industrial cultivation. The strains should develop well in the food matrix and maintain a high concentration of active cells in the finished food products during both production and storage. This result prompted the need to cultivate the selected strains of *Lactobacillus delbrueckii ssp. bulgaricus* in reconstituted skim milk powder and under static conditions. Mathematical modeling of *Lactobacillus* growth kinetics was also conducted.

It was known that an increase in the concentration of lactic acid in the medium resulted in a certain inhibiting action on the growth of lactobacilli and that this sensitivity to lactic acid was strain-specific (Bouguettoucha et al. 2011; Gordeev et al. 2017). For this reason, the selected mathematical models included parameters characterizing the effect of lactic acid on lactobacilli.

Mathematical models for the kinetics of fermentation processes:

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

Model 1

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

Model 2

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

Model 3

$$K_T = a - b e^{-(q_p \tau)^\delta}$$

Weibull

where: μ_{max} is the maximum specific growth rate, h^{-1} ; X_b , P_b , X_{bm} and P_{bm} are the biomass, the amount of lactic acid, the final concentration of biomass and lactic acid in a dimensionless form; c - a parameter measuring the inhibitory effect of the accumulated product (lactic acid) on cell development; n and n_1 - coefficients that measure the influence of lactic acid on the cells, respectively showing the resistance of the cells to the increasing concentration of the product; q - a coefficient indicating the inhibitory action of the product, respectively, of lactic acid on its own synthesis; K_T - titratable acidity in dimensionless form; a - maximum value of titratable acidity in a dimensionless form; b - a coefficient equal to the difference between the maximum and the initial titratable acidity in a dimensionless form; q_p - specific rate of acid formation, h^{-1} ; δ - an indicator defining the change in the shape of the curve or the change in the rate of accumulation of lactic acid over time; τ - time of cultivation, h^{-1} .

When studying and comparing the kinetics of development in individual strains in the same cultivation environment and under the same conditions, it is necessary to start the process with the same concentration of active cells and the same initial acidity. Because this is challenging, the biomass and titratable acidity in mathematical models were presented in a non-dimensional form (Tishin and Fedorov 2016; Tishin and Golovinskaya 2015). The model parameters were identified, and the results are presented in Table 3 which shows that the selected models are characterized by relatively high correlation coefficients (from 0.9664 to 0.9924 for different models). A comparison of the experiment data with the models was made and the results are presented in Fig. 1 through 4.

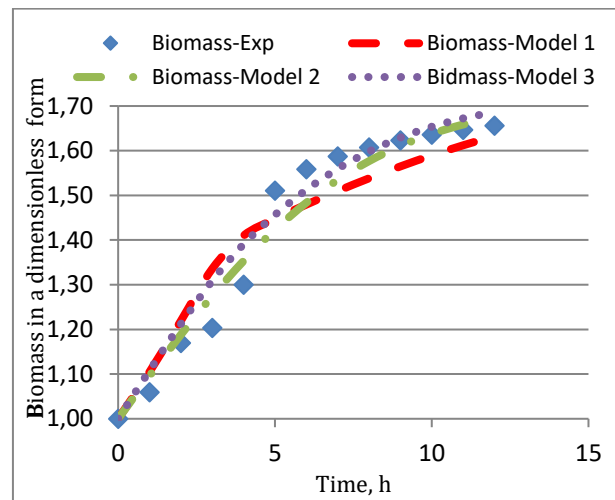


Figure 1. Comparison of experimental data with those of selected models for *L. bulgaricus* MG 1

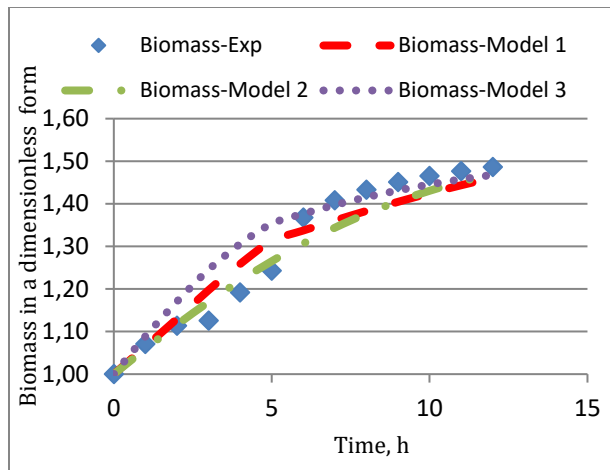


Figure 2. Comparison of experimental data with those of selected models for *L. bulgaricus* MG 2

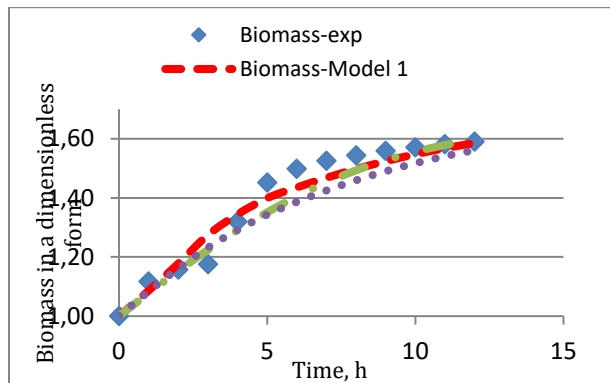


Figure 3. Comparison of experimental data with those of selected models for *L. bulgaricus* MG 3

These figures show that the patterns were well aligned with the study results. From the data presented in the table 3 it is evident that in the MG3 strain there was a relatively higher inhibitory effect of lactic acid on the maximum specific growth rate. For this strain, the "c" coefficient in model 1, showing the degree of lactic acid influence on strain development, was highest (2,028) compared to the other strains studied. However, strain MG3 developed at a relatively high growth rate of 0.077 h⁻¹. For strains MG1 and MG4, the coefficient "c" had commensurate values, indicating that lactic acid would have an almost equal influence on the development of both strains. For these lactobacilli, parameter "c" took lower values (1,360 and 1,513)

compared to strain MG3, indicating that lactic acid would have a lesser effect on the development of the strains studied. According to model 1, the maximum growth rate for these two strains was 0.088 and 0.047 h⁻¹. The lowest influence of cumulative lactic acid was observed for strain MG2.

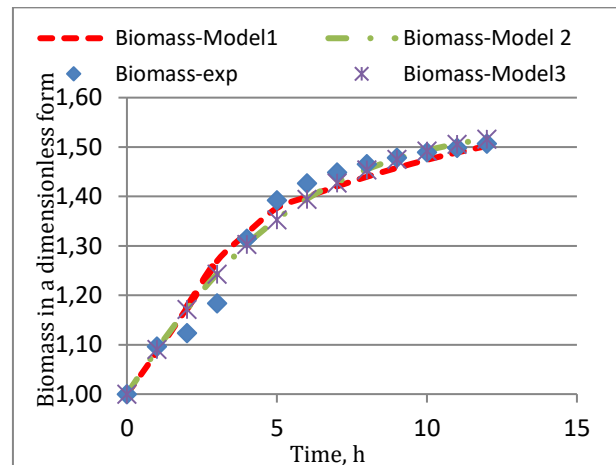


Figure 4. Comparison of experimental data with those of selected models for *L. bulgaricus* MG 4

For this strain, parameter "c" took the lowest values (1,056) but still developed at a lower maximum specific growth rate (0,047 h⁻¹), which was related to the marginal capabilities of this strain. According to the data shown in Table 3, Model 2 produced higher values for the maximum specific growth rate for all strains tested with numbers at - 0.125, 0.088, 0.101 and 0.160 h⁻¹, respectively. In Model 2, parameter "n" indicates the influence of lactic acid on the biomass itself or the resistance of the microbial cells to the accumulation of lactic acid. From the results in Table 3 it is evident that the lowest values for this coefficient (0.854) were observed for MG3. This result would mean that this strain was less sensitive to the amount of lactic acid than the other strains and that it is essential to maintain a high number of active cells in the finished milk product during storage. It is these cells that give the finished product its beneficial effect. MG2 demonstrated strain resistance to lactic acid that was closer to the resistance shown by the preceding strain. For MG2, the parameter "n" took a value of 0.891. The highest sensitivity to lactic acid was observed for MG4, followed by MG1.

Table 3. Kinetic parameters in Models 1, 2 and 3 for *Lactobacillus bulgaricus*

Strain	Mathematical model												
	Model 1				Model 2				Model 3				
	μ_m, h^{-1}	c	R ²	e	μ_m, h^{-1}	n	R ²	e	μ_m, h^{-1}	n ₁	q	R ²	e
<i>L. bulgaricus</i> MG1	0,080	1,360	0,9805	0,042	0,125	0,914	0,9811	0,043	0,134	0,822	0,175	0,9805	0,042
<i>L. bulgaricus</i> MG2	0,047	1,056	0,9749	0,032	0,088	0,891	0,9845	0,034	0,130	0,733	0,750	0,9664	0,031
<i>L. bulgaricus</i> MG3	0,077	2,028	0,9723	0,038	0,101	0,854	0,9764	0,040	0,089	0,534	0,445	0,9684	0,039
<i>L. bulgaricus</i> MG4	0,063	1,531	0,9895	0,031	0,160	1,088	0,9924	0,032	0,162	1,093	0,007	0,9924	0,032

Table 4. Kinetic parameters in the Weibull model

Strain	a,	b,	q_p, h^{-1}	δ	R ²	e
<i>L. bulgaricus</i> MG1	5,95	4,87	0,136	1,14	0,9724	0,354
<i>L. bulgaricus</i> MG2	4,97	4,12	0,086	0,909	0,9553	0,301
<i>L. bulgaricus</i> MG3	6,36	5,58	0,096	1,07	0,9822	0,186
<i>L. bulgaricus</i> MG4	6,56	5,97	0,179	1,35	0,9664	0,348

Table 5. Weibull's mathematical model in explicit form

Strain	Explicit models
<i>L. bulgaricus</i> MG1	$K_T = 5,95 - 4,87e^{-(0,136\tau)^{1,14}}$
<i>L. bulgaricus</i> MG2	$K_T = 4,97 - 4,12e^{-(0,086\tau)^{0,91}}$
<i>L. bulgaricus</i> MG3	$K_T = 6,36 - 5,58e^{-(0,096\tau)^{1,07}}$
<i>L. bulgaricus</i> MG4	$K_T = 6,56 - 5,97e^{-(0,179\tau)^{1,35}}$

For these strains, the parameter "n" was 1.088 and 0.914, respectively. This result would imply the efficacy of these strains in the efficacy of these strains in the composition of starter culture for milk with a higher buffering capacity in order to reduce the effects of the accumulated lactic acid. In Model 3, the parameter "n₁" has the same meaning as in the previous model. Again, according to this model, MG3 was the most resistant to lactic acid. For MG3, "n₁" took the relatively low value of 0.534 which again confirmed that this strain was expected to retain a higher amount of active cells during storage of the finished product. In this model, MG3 had a lower growth rate than the other strains (0.089 h⁻¹). Model 3 demonstrated that the next in line for resistance to lactic acid was strain MG2 for which parameter "n₁" took a value of 0.733. The test strain also had a relatively high growth rate of 0.130 h⁻¹. According to Model 3 data, MG4 exhibited the highest susceptibility to lactic acid, followed by MG1. For these strains, the coefficient "n₁" took values of 1,093 and 0,822, respectively. However, MG4 had the highest maximum specific growth rate of 0.162 h⁻¹. Strain MG1 was developed with a maximum specific growth rate (0,134 h⁻¹) that was close to that of MG2. From the mathematical modeling performed, it can be assumed that strains with higher resistance to lactic acid will have a greater survival rate when subjected to the low pH values of the stomach. Another important parameter from a technology point of view is parameter "q" in Model 3. This parameter shows the inhibitory action of the product of lactic acid on its own synthesis. The lowest value for this coefficient was observed for MG4 (0.007) and characterized the strain with increased acid-formation as well as the highest post-acidification ability compared to the other strains. This higher post-acidification ability would invariably lead to unnecessary acidification of the finished product during storage. A similar trend was observed for strain MG1. In this case, "q" took a value of 0.175. Lower values of parameter "q" were observed in strains MG2 and MG3 at 0.750 and 0.445, respectively. These results would indicate that the last two strains are less acid-forming and that much lower post-acidic formation would be expected during storage of products containing strains MG2 and MG3. In order to confirm our

assumptions, the specific acidity rate q_p and the degree of change in the intensity of lactic acid accumulation over time as a whole (δ) were calculated. The results of the modeling carried out are presented in Tables 4 and 5. The results presented in Table 4 show that strains MG4 and MG1 had significantly higher specific acidity rates (0.179 and 0.136 h⁻¹) compared to the other two strains, (which had specific acidity rates of 0.086 and 0.096 h⁻¹, respectively). Also noteworthy are the higher values of parameter δ for strains MG4 and MG1 (1.35 and 1.17). Strains MG2 and MG3 exhibited lower growth rates, respectively 0.086 h⁻¹ and 0.096 h⁻¹. A similar trend was observed for the parameter δ (0.91 and 1.07). This trend would indicate that in these strains the acidification progressed more moderately and did not exhibit pronounced post-acid-forming ability.

Conclusions

Our examination of the antimicrobial activity and acid-forming ability of *Lactobacillus delbrueckii* ssp. *bulgaricus* strains provided following results. Strains MG4 and MG1 were developed as shown by Models 2 and 3 with a higher maximum growth rate compared to strains MG2 and MG3. Strains MG4 and MG1 exhibited a more pronounced sensitivity to the accumulated lactic acid as evidenced by the higher values of parameters 'n' and 'n₁' compared to strains MG2 and MG3. This result would support the use of these particular strains as starter cultures in the fermentation of milk with a higher buffering capacity. Strains MG4 and MG1 had a stronger acid-forming ability compared to strains MG2 and MG3. MG4 and MG1 showed a high maximum rate of acid formation and low values of parameter 'q₁' in Model 3. This would indicate that MG4 and MG1 are more likely to exhibit significant post-acid-forming ability and unnecessary acidification of the finished products during storage. Strains MG2 and MG3 were moderate acidic forming strains, as the maximum acidity rate and coefficient 'δ' were balanced. These strains also showed high values for parameter 'q' in model 3. This would indicate that the strains tend to have a lower post-acid-forming ability and are therefore less prone to excessive acidification of the finished products during storage.

As a result of this study and mathematical modeling, we concluded that the MG3 strain is the recommended one to increase the efficiency of starter cultures used in dairy production. MG3 had the highest antimicrobial activity along with susceptibility to a greater number of antibiotics. As a result, the cells of this strain had the highest resistance to the accumulation of lactic acid and possessed moderate acid-forming ability and low post-acidification.

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