***In vitro* examination of the antimicrobial activity of a potentially probiotic *Lactobacillus acidophilus* strain against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* sp.**

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Abstract. An interesting property for many probiotic strains is to exhibit antimicrobial activity against pathogenic microorganisms. Therefore, the antimicrobial activity of the potentially probiotic strain *Lactobacillus acidophilus* L12 against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* sp. was examined *in vitro* through co-culturing of *Lactobacillus acidophilus* L12 with each of the pathogenic strains for 72 h. When cultured individually, all strains reached a final population always exceeding 10^{12} cfu/mL. When co-cultured with each of the pathogenic strains *Lactobacillus acidophilus* L12 population always exceeded 10^{12} cfu/mL, while the bacterial population of the pathogenic strains was reduced in a strain-specific manner. *Lactobacillus acidophilus* L12 demonstrated the highest inhibitory activity against the two *Staphylococcus aureus* strains, followed by the two *E. coli* strains, and the two *Salmonella* sp. strains tested. The kinetics of the co-culturing process of *Lactobacillus acidophilus* L12 with each of the test pathogenic strains was modelled using the logistic curve model. The obtained results show the strong antagonistic activity of the potentially probiotic strain *Lactobacillus acidophilus* L12, thus making it a promising candidate for inclusion in the composition of probiotic preparations and functional probiotic foods.

1 Introduction

Only lactobacilli strains that have certain properties can be included in the composition of probiotics and probiotic foods [1]: (i) to be part of the natural microflora in humans and animals; (ii) to have the ability to adhere to epithelial cells or cell lines; (iii) to survive in the conditions of the stomach and intestines, i.e. to survive in the conditions of acidic pH in the stomach and to be resistant to the action of bile juice; (iv) to reproduce in the gastrointestinal tract by assimilating mainly the substrate and to suppress and expel from the biological niche pathogenic and toxigenic microorganisms; (v) to allow industrial cultivation, inclusion in gel matrices (encapsulation) and to retain their activity following processes such as freeze-drying, as well as in the finished products; (vi) to have antimicrobial activity against opportunistic, carcinogenic and pathogenic microorganisms, which is associated with inactivation of their enzyme systems, overcoming their adhesion, inhibiting their growth and/or expulsion from the biological niche, resulting in normalization of the gastrointestinal microflora; (vii) to produce antimicrobial

substances; (viii) to modulate the immune response; (ix) to be safe for clinical and nutritional use.

Thus, an interesting property for probiotic strains is to exhibit antimicrobial activity against pathogenic microorganisms so that they can play their antagonistic role in the human gastrointestinal tract and reduce the number of harmful bacteria and restore the balance of the gastrointestinal microflora [2, 3].

Lactobacillus acidophilus strains have already been reported to adhere to the surface of intestinal epithelial cells. However, this adhesion is strictly specific to each individual [4]. Adhesion is performed with lectin-like proteins in the surface protein layer of *Lb. acidophilus*. Besides, several *Lactobacillus acidophilus* strains produce bacteriocins such as lactacin B, lactacin F and acidocin. The bacteriocin gaseicin A was isolated from *Lactobacillus gasseri*. It shows high inhibitory activity against a wide range of food pathogenic bacteria such as *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus*. Gaseicin A is a highly hydrophobic bacteriocin. This is the first cyclic bacteriocin isolated from the group of lactic acid bacteria [5, 6].

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The aim of the present study was to determine the *in vitro* inhibitory activity of a potential probiotic strain of *Lactobacillus acidophilus* against the growth of the pathogenic bacterial strains *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 25293, *S. aureus* ATCC 6538P, *Salmonella enteritidis* ATCC 13076, and *Staphylococcus abony* NTCC 6017, by co-culturing the *Lactobacillus acidophilus* L12 strain with each of these pathogenic bacterial strains.

2 Materials and methods

2.1 Microorganisms

The studies in the present work were performed with *Lactobacillus acidophilus* L12 isolated from the rose blossom of *Rosa damascena* Mill and identified by the authors of the present manuscript using biochemical (API 50 CHL) and molecular-genetic (sequencing of the 16S rDNA) methods (data not published). The strain is stored at $4 \pm 2^\circ\text{C}$ or frozen.

The antagonistic activity against 6 foodborne pathogenic bacterial strains of *Lactobacillus acidophilus* L12 strain was investigated: *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 25293, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus enteritidis* ATCC 13076, *Staphylococcus abony* NTCC 6017.

2.2 Media

2.2.1 MRS broth

Composition (g/l): peptone from casein – 10; yeast extract – 4; meat extract – 8; glucose – 20; K_2HPO_4 – 2; sodium acetate – 5; diammonium citrate – 2; MgSO_4 – 0.2; MnSO_4 – 0.04; Tween 80-1 ml/l; pH = 6.5. Sterilization – 15 minutes at 118°C .

2.2.2 LAPTg10 broth

Composition (g/l): peptone – 15; yeast extract – 10; tryptone – 10; glucose – 10; Tween 80-1 ml/l. pH=6.6 – 6.8. Sterilization – 20 min at 121°C .

2.2.3 LAPTg10 agar

Composition (g/l): peptone – 15; yeast extract – 10; tryptone – 10; glucose – 10; Tween 80-1 ml/l, agar – 15. pH = 6.6 – 6.8. Sterilization – 20 min at 121°C .

2.2.4 LBG agar

Composition (g/l): tryptone – 10; yeast extract – 5; NaCl – 10; glucose – 10; agar – 15; pH = 7.5. Sterilization – 20 min at 121°C .

3. Methods of analysis

3.1. Determination of the antimicrobial activity of *Lactobacillus acidophilus* L12 against pathogenic microorganisms - by co-culturing

To determine the antimicrobial activity of the studied strain against pathogenic bacteria, a 24h culture of *Lactobacillus acidophilus* L12 (the concentration of viable cells being 10^9cfu/ml) was used. 0.5 mL of the lactobacilli suspension was mixed with 0.5 mL of the pathogen suspension (the concentration of viable cells being 10^9cfu/ml) and 9 mL of culture medium (MRS broth or LAPTg10 broth). For the *Lactobacillus acidophilus* L12 control as well as for the pathogen controls 9.5 mL of the respective culture medium was mixed with 0.5 mL of the lactobacilli suspension or the pathogen suspension, respectively. Co-cultivation of lactobacilli and pathogenic bacteria was performed under static conditions at 37°C for 60 to 72h, taking samples at 0, 12, 24, 36, 48, 60, and 72 h and monitoring the change in the titratable acidity and the concentration of viable cells of both the pathogen and *Lactobacillus acidophilus* L12 [7].

3.2. Determination of the titratable acidity

Torner's method was used to determine the acid-forming ability of lactic acid bacteria. One °T equals 1 mL of 0.1 N NaOH used to neutralize an equivalent amount of organic acid contained in 100 mL of culture medium.

The method was based on titration of the sample with 0.1 N NaOH. For this purpose, 10 mL of each sample were mixed with 20 mL of distilled water. The mixture was titrated with 0.1N NaOH using phenolphthalein as an indicator until the appearance of a light pink color that persists for one minute [8].

3.3 Determination of the number of viable cells of lactobacilli and pathogenic microorganisms

Appropriate tenfold dilutions of each sample were prepared and used for spread-plating on LAPTg10-agar (for the enumeration of lactobacilli) and on LBG-agar (for the enumeration of pathogenic bacteria). The inoculated Petri dishes were cultured for 3 days at 37°C until the appearance of countable single colonies. The number of the colonies was used to estimate the number of viable cells in the original sample [7].

3.4. Determination of kinetic parameters

The classical (1) and modified models of the logistic curve (2) were used to model the growth kinetics. Since the amount of pathogens killed per time unit as a result of the antimicrobial metabolites released by the lactobacilli (lactic acid and other organic acids and bacteriocins) is proportional to the initial amount of living cells, an equation describing the kinetics of first-order chemical reactions was used to describe the death kinetics (3).

$$\begin{aligned}\frac{dX}{d\tau} &= \mu X - \beta X^2 \\ &\Rightarrow \mu X - \frac{\mu}{X_k} X^2 \\ &\Rightarrow \mu \left(1 - \frac{X}{X_k}\right) X\end{aligned}\quad (1)$$

$$\begin{aligned}\frac{dX}{d\tau} &= (\mu X - \beta X^2)^n \\ &\Rightarrow \left(\mu X - \frac{\mu}{X_k} X^2\right)^n \\ &\Rightarrow \mu \left(1 - \frac{X}{X_k}\right)^n X\end{aligned}\quad (2)$$

$$\frac{dX}{d\tau} = -kX \Rightarrow X = X_0 e^{-k\tau} \quad (3)$$

Where: μ is the maximum specific growth rate, h^{-1} ; X_0 , X and X_k are the initial, current and final biomass concentrations, respectively, cfu/mL; β - coefficient of internal population competition, cfu/mL.h; k - death rate, h^{-1} ; n - parameter, describing the influence of the composition of the medium and the metabolic products released by the studied microorganisms (lactobacilli and pathogens) on the cells and their growth; τ - time, h^{-1} .

The differential equations were solved by the 4th order Runge-Kutta method [9] in Excel. The general and discretized type of the equation are:

$$\begin{aligned}\frac{dy}{dx} &= F(x, y) \\ \frac{y_{i+1} - y_i}{x_{i+1} - x_i} &= \frac{y_{i+1} - y_i}{h} = F(x, y)\end{aligned}$$

Where h is step of the solution.

For a system of ordinary differential equations ($k = 1, \dots, K$ equations):

$$\begin{aligned}\frac{dy_1}{dx_1} &= F_1(x, y_1, y_2, \dots, y_k) \\ \frac{dy_2}{dx_2} &= F_2(x, y_1, y_2, \dots, y_k) \\ \frac{dy_k}{dx_k} &= F_k(x, y_1, y_2, \dots, y_k)\end{aligned}$$

The algorithm for solving ordinary differential equations by the method of Runge-Kutta of the 4th order, is the following:

$$\begin{aligned}y_{k,i+1} &= y_{k,i} + \frac{1}{6}(k_{k,1} + 2 \cdot k_{k,2} + 2 \cdot k_{k,3} + k_{k,4}) \\ k_{k,2} &= hF_k\left(x_i + \frac{h}{2}, y_{k,i} + \frac{k_{k,1}}{2}\right) \\ k_{k,1} &= hF_k(x_i, y_{1,i}, y_{2,i}, \dots, y_{k,i}) \\ k_{k,3} &= hF_k\left(x_i + \frac{h}{2}, y_{k,i} + \frac{k_{k,2}}{2}\right) \\ k_{k,4} &= hF_k(x_i + h, y_{k,i} + k_{k,3})\end{aligned}$$

The Solver function in Excel was used to identify the parameters of the models. The model parameters were determined by minimizing the square of the difference between the experimental data and those obtained from the respective model.

3.5 Processing the results

Data from triplicate experiments were processed with MS Office Excel 2010 software products, using statistical functions to determine the standard deviation and the maximum estimation error at significance levels of $p < 0.05$.

4 Results and discussion

Lactobacillus strains are increasingly included in the composition of probiotics and functional foods. Several *Lactobacillus acidophilus* strains are Generally Recognized as Safe by the FDA and benefit from a Qualified Presumption of Safety status in EU., *Lb. acidophilus* bacteria are thus included in a wide range of probiotic preparations and probiotic foods marketed. A study is necessary to determine the probiotic potential of *Lactobacillus acidophilus* L12.

The antimicrobial activity of the potentially probiotic *Lactobacillus acidophilus* L12 strain against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* sp. was examined *in vitro* through co-culturing with each of the 6 pathogenic bacterial strains tested for 72 h, monitoring the changes in the number of viable cells of the probiotic strain and the pathogenic strains as well as the changes in the titratable acidity. All strains reached high number of viable cells after 72 h separate culturing (Fig. 1a, Fig. 2a, Fig. 3a, Fig. 4a, Fig. 5a, Fig. 6a). *Lactobacillus acidophilus* L12 always reached a high concentration of viable cells during its co-culturing with each of the test pathogenic strains, while the concentration of viable cells of the pathogenic strains was reduced in a strain-specific manner (Fig. 1a, Fig. 2a, Fig. 3a, Fig. 4a, Fig. 5a, Fig. 6a).

During the separate growth of *Lactobacillus acidophilus* L12 under static conditions for 12-24 h, the

titratable acidity of the medium reached 90°T (Fig. 1b, Fig. 2b).

Fig. 1a. Monitoring of the number of viable cells of *Lb. acidophilus* L12 and *E. coli* ATCC 25922 during separate culturing and co-culturing at 37±1°C.

Fig. 1b. Monitoring of the titratable acidity of the medium during separate culturing and co-culturing of *Lb. acidophilus* L12 and *E. coli* ATCC 25922 at 37±1°C.

Fig. 2a. Changes in the number of viable cells of *Lb. acidophilus* L12 and *E. coli* ATCC 8739 during separate culturing and co-culturing at 37±1°C.

Fig. 2b. Changes in the titratable acidity of the medium during separate culturing and co-culturing of *Lb. acidophilus* L12 and *E. coli* ATCC 8739 at 37±1°C.

In the co-culturing of *Lactobacillus acidophilus* L12 and *Escherichia coli* ATCC 25922 at 37±1°C under static conditions, an increase in the concentration of viable cells of *Escherichia coli* ATCC 25922 was observed during the first 36 h, followed by gradual decrease, reaching almost 0 after 72 h (Fig. 1a).

When *Lactobacillus acidophilus* L12 was co-cultured with *Escherichia coli* ATCC 8739 at 37±1°C under static conditions, there was an increase in the number of living cells of the pathogen during the first 36 h. Then, a sharp decrease in the number of living cells of *Escherichia coli* ATCC 8739 began, and no viable cells of the pathogenic microorganism were identified after 72 h (Fig. 2a).

The established inhibition of the pathogens by the lactobacilli strain in the mixtures might be due to the increase in the titratable acidity due to the accumulation of lactic acid and other organic acids, as well as the production of peptide metabolites such as bacteriocins with antimicrobial action in the medium.

In a mixed population of *Lactobacillus acidophilus* L12 and *Salmonella* sp., the concentration of viable pathogen cells began to decrease after 36 h and after 72 h there were about 10³ cfu/ml living cells of *Salmonella abony* NTCC 6017. In the co-culturing of *Lactobacillus acidophilus* L12 and *Salmonella enteritidis* ATCC 13076 the reduction of viable pathogen cells began after 24 h and after 72h no living pathogenic bacteria were detected, while for the same time interval the concentration of lactobacilli reached above 10¹² cfu/mL (Fig. 3a, Fig. 4a).

When examining the antagonistic activity of *Lactobacillus acidophilus* L12 against *Staphylococcus aureus* ATCC 25293 and *Staphylococcus aureus* ATCC 6538P, there was an increase in the concentration of viable cells of both pathogens during the first 24 h, followed by a decrease and the number of living pathogens by the 72nd h was less than the detection threshold which was 10cfu/mL (Fig. 5a, Fig. 6a).

Fig. 3a. Changes in the number of viable cells of *Lb. acidophilus* L12 and *S. abony* NTCC 6017 during separate culturing and co-culturing at 37±1°C.

Fig. 3b. Changes in the titratable acidity of the medium during separate culturing and co-culturing of *Lb. acidophilus* L12 and *S. abony* NTCC 6017 at 37±1°C.

Fig. 4a. Changes in the number of viable cells of *Lb. acidophilus* L12 and *S. enteritidis* ATCC 13076 during separate culturing and co-culturing at 37±1°C.

As expected, while titratable acidity increased over time when *Lactobacillus acidophilus* L12, which is a lactic acid bacterium, was inoculated unlike when pathogenic bacteria were inoculated alone. (Fig. 1b, Fig. 2b, Fig. 3b, Fig. 4b, Fig. 5b, Fig. 6b).

Acidification of broth leads to a change in the conditions for the growth of the pathogenic bacteria.

The inhibition of their growth by the *Lactobacillus plantarum* strain was thus likely largely due to the reduced pH and to the formation and accumulation of lactic acid and other organic acids. However, it could also result from the synthesis of other antimicrobial

metabolites by *Lactobacillus acidophilus* L12, such as bacteriocins.

Fig. 4b. Changes in the titratable acidity of the medium during separate culturing and co-culturing of *Lb. acidophilus* L12 and *S. enteritidis* ATCC 13076 at 37±1°C.

Fig. 5a. Changes in the number of viable cells of *Lb. acidophilus* L12 and *S. aureus* ATCC 6538P during separate culturing and co-culturing at 37±1°C.

Fig. 5b. Changes in the titratable acidity of the medium during separate culturing and co-culturing of *Lb. acidophilus* L12 and *S. aureus* ATCC 6538P at 37±1°C.

In order to clarify the interaction of pathogens and lactobacilli in their co-cultivation, it is important to monitor the changes in their maximum specific growth rates.

An important kinetic parameter is the pathogen death rate and its reciprocal value, which shows the resistance of the pathogen to the created adverse conditions.

Table 1 shows the kinetic parameters for lactobacilli and pathogens in separate culturing and in co-culturing.

Fig. 6a. Changes in the number of viable cells of *Lb. acidophilus* L12 and *S. aureus* ATCC 25923 during separate culturing and co-culturing at $37 \pm 1^\circ\text{C}$.

Fig. 6b. Changes in the titratable acidity of the medium during separate culturing and co-culturing of *Lb. acidophilus* L12 and *S. aureus* ATCC 25923 at $37 \pm 1^\circ\text{C}$.

In the co-culturing of *Lactobacillus acidophilus* L12 and the pathogens *Staphylococcus abony* NTCC 6017, *S. enteritidis* ATCC 13076, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus aureus* ATCC 25293, *Escherichia coli* ATCC 25922 and *Escherichia coli* ATCC 8739, the maximum specific growth rate of *Lactobacillus acidophilus* L12 decreased, but yet it retained relatively high values. According to the two logistic models, it varied in the range from 0.026 to 0.038 h^{-1} (compared to its value in separate culturing that varied between 0.040 h^{-1} and 0.042 h^{-1}). There was also an increase in the parameter n , which ranged from 0.9917 to 1.1761, which is a result of the competition of lactobacilli and pathogens for nutrients. In addition, the increase in the value of the parameter n shows certain influence of the metabolites released by the pathogens on the growth of *Lb. acidophilus* L12. Nevertheless, high values of the maximum concentration of active cells of *Lactobacillus acidophilus* L12, which would theoretically be reached in the mixed population, were observed. According to the models used, X_k had values close to the control (separate culturing of the strain) and varied in the range from 12.10 log N to 13.03 log N.

In the co-culturing of *Staphylococcus aureus* ATCC 25293 and *S. aureus* ATCC 6538P with *Lactobacillus acidophilus* L12, a reduction in the maximum specific growth rate of the pathogens was determined. According to the models used, μ decreased from 0.089 h^{-1} and 0.096

h^{-1} to 0.062 h^{-1} and 0.051 h^{-1} for *Staphylococcus aureus* ATCC 25293, while μ decreased from 0.085 h^{-1} and 0.099 h^{-1} to 0.051 h^{-1} and 0.070 h^{-1} for *Staphylococcus aureus* ATCC 6538P. With the two strains studied, the parameter n increased, having values above 1. This parameter was 1.0486 and 1.0265 for *Staphylococcus aureus* ATCC 25293 and *Staphylococcus aureus* ATCC 6538P, respectively. This indicated that the pathogen growth was influenced by the presence of the lactobacilli and their metabolites with antimicrobial activity. This fact was confirmed by the reduction of the maximum final concentration of active cells of the studied pathogen, which would theoretically be reached in the mixed population. X_k for *Staphylococcus aureus* ATCC 25293 was 10.30 log N and 10.96 log N, depending on the model, while X_k for *Staphylococcus aureus* ATCC 6538P was 12.48 log N and 12.80 log N. Relatively high and comparable values of the death rate constant were observed for the two *S. aureus* strains - 0.615 h^{-1} and 0.608 h^{-1} for *S. aureus* ATCC 25293 and *Staphylococcus aureus* ATCC 6538P, respectively.

In the co-cultivation of *Staphylococcus abony* NTCC 6017 and *Lb. acidophilus* L12, a reduction in the maximum specific growth rate of the studied pathogen was observed. According to the two logistics models for *Staphylococcus abony* NTCC 6017 μ was 0.068 h^{-1} and 0.075 h^{-1} . According to the modified logistics model, the reduction in the maximum specific growth rate of the pathogen was significant, compared to the control, where μ had a 0.278 h^{-1} value. With *Staphylococcus abony* NTCC 6017, a certain increase in the parameter n was observed. It was 0.9842 (compared to the control - 0.8909). This indicated that the pathogen would resist the conditions created by the presence of the lactobacilli and their metabolites longer. For *Staphylococcus abony* NTCC 6017, the two logistic models predicted by almost one logarithmic unit a lower value of X_k , and the value according to the models was 10.83 log N and 10.91 log N (compared to the control, where X_k was 11.57 log N). This strain has the lowest value of the death rate compared to all other pathogens studied. For *Staphylococcus abony* NTCC 6017 it was 0.322 h^{-1} .

A reduction in the maximum specific growth rate by almost 50% for *Staphylococcus enteritidis* ATCC 13076 was observed.

The μ values for this strain were 0.034 h^{-1} and 0.046 h^{-1} , compared to the control (0.065 h^{-1} and 0.077 h^{-1}).

The parameter n increased for *Staphylococcus enteritidis* ATCC 13076, and its value was 1.0248, which in turn indicated that the strain was affected by the presence of the lactobacilli and their metabolites. In the co-cultivation of *Staphylococcus enteritidis* ATCC 13076 and *Lactobacillus acidophilus* L12, a significant decrease in the maximum concentration of active cells of the pathogen, which would theoretically be reached in the mixed population - 11.64 log N and 11.63 log N (compared to the control - 14.37 log N and 14.29 log N), was observed. This pathogen had a relatively high death rate - 0.535 h^{-1} .

Table 1. Kinetic parameters.

Strain/Mixture	Kinetic parameters							
	Model 1			Model 2				Model 3
	μ , h^{-1}	β , cfu/mL.h	X_K , cfu/mL	μ , h^{-1}	β , cfu/mL.h	X_K , cfu/mL	n	k, h^{-1}
<i>Lb. acidophilus</i> L12 κ	0.040	0.0030	13.07	0.042	0.0032	13.14	0.8990	-
<i>S. abony</i> NTCC 6017 κ	0.077	0.0066	11.57	0.278	0.0240	11.57	0.9419	-
<i>S. enteritidis</i> ATCC 13076 κ	0.065	0.0045	14.37	0.077	0.0054	14.29	0.8909	-
<i>S. aureus</i> ATCC 25923 κ	0.089	0.0065	13.68	0.096	0.0064	14.96	0.9064	-
<i>S. aureus</i> ATCC 6538P κ	0.085	0.0063	13.46	0.099	0.0071	13.96	0.9641	-
<i>E. coli</i> ATCC 25922 κ	0.099	0.0074	13.33	0.086	0.0064	13.35	0.9017	-
<i>E. coli</i> ATCC 8739 κ	0.096	0.0070	13.85	0.097	0.0073	13.35	0.9010	-
<i>Lb. acidophilus</i> L12 (<i>Lb. acidophilus</i> L12 + <i>S. abony</i> NTCC 6017)	0.029	0.0024	12.49	0.033	0.0025	13.03	1.0988	-
<i>Lb. acidophilus</i> L12 (<i>Lb. acidophilus</i> L12 + <i>S. enteritidis</i> ATCC 13076)	0.036	0.0028	12.89	0.034	0.0026	12.80	0.9917	-
<i>Lb. acidophilus</i> L12 (<i>Lb.</i> <i>acidophilus</i> L12 + <i>S. aureus</i> ATCC 6538P)	0.032	0.0020	12.82	0.038	0.0030	12,65	0.9940	-
<i>Lb. acidophilus</i> L12 (<i>Lb. acidophilus</i> L12 + <i>S. aureus</i> ATCC 25923)	0.027	0.0021	12.44	0.036	0.0028	12.68	1.1761	-
<i>Lb. acidophilus</i> L12 (<i>Lb. acidophilus</i> L12 + <i>E. coli</i> ATCC 25922)	0.026	0.0021	12.10	0.027	0.0022	12.44	1.1350	-
<i>Lb. acidophilus</i> L12 (<i>Lb. acidophilus</i> L12 + <i>E. coli</i> ATCC 8739)	0.025	0.0020	12.89	0.038	0.0030	12.83	1.0996	-
<i>S. abony</i> NTCC 6017 (<i>Lb. acidophilus</i> L12 + <i>S.</i> <i>abony</i> NTCC 6017)	0.068	0.0063	10.83	0.075	0.0069	10.91	0.9842	0.322
<i>S. enteritidis</i> ATCC 13076 (<i>Lb. acidophilus</i> L12 + <i>S.</i> <i>enteritidis</i> ATCC 13076)	0.034	0.0029	11.64	0.046	0.0040	11.63	1.0248	0.535
<i>S. aureus</i> ATCC 25923 (<i>Lb. acidophilus</i> L12 + <i>S.</i> <i>aureus</i> ATCC 25923)	0.062	0.0060	10.36	0.051	0.0046	10.96	1.0486	0.615
<i>S. aureus</i> ATCC 6538P (<i>Lb. acidophilus</i> L12 + <i>S.</i> <i>aureus</i> ATCC 6538P)	0.058	0.0047	12.48	0.070	0.0055	12.80	1.0265	0.608
<i>E. coli</i> ATCC 25922 (<i>Lb. acidophilus</i> L12 + <i>E. coli</i> ATCC 25922)	0.071	0.0068	10.47	0.078	0.0075	10.52	1.0121	0.652
<i>E. coli</i> ATCC 8739 (<i>Lb. acidophilus</i> L12 + <i>E. coli</i> ATCC 8739)	0.031	0.0026	12.17	0.054	0.0045	12.18	1.0860	0.741

In the co-culturing of *Escherichia coli* ATCC 25922 or *Escherichia coli* ATCC 8739 and *Lactobacillus acidophilus* L12, a reduction in the maximum specific growth rate was determined. The μ value for *Escherichia coli* ATCC 25922 was 0.071 h^{-1} and 0.078 h^{-1} (compared to the control for this strain in which μ was 0.099 h^{-1} and 0.086 h^{-1}). The parameter n for this strain was 1.0121 (compared to the control, in which n was 0.9017), which showed that the growth of *Escherichia coli* ATCC 25922 was influenced by the presence of the lactobacilli and their metabolites with antimicrobial action. This was confirmed by the lower value of the maximum final concentration in the mixed population, which would theoretically reach $10.47\log N$ and $10.52\log N$. With this strain there was a relatively high death rate - 0.652 h^{-1} .

A reduction in the maximum specific pathogen growth rate was also observed in the mixed population of *Escherichia coli* ATCC 8739 and *Lactobacillus acidophilus* L12. The μ value for *Escherichia coli* ATCC 8739 was 0.031 h^{-1} and 0.054 h^{-1} .

The maximum final biomass concentration of *Escherichia coli* ATCC 8739 that would theoretically be reached in the mixed population, calculated according to the two models, was $12.17\log N$ and $12.18\log N$, which was almost one logarithmic unit lower than that of the control ($13.33\log N$ and $13.35\log N$). The death rate constant for *Escherichia coli* ATCC 8739 was 0.741 h^{-1} .

The obtained results regarding the antimicrobial activity of the newly isolated strain with probiotic potential *Lactobacillus acidophilus* L12 against pathogenic microorganisms unequivocally showed that the strain demonstrated high inhibitory activity and a high degree of reduction of the viable cells of the studied pathogens. This is promising for the selection for inclusion in the composition of probiotics and functional foods.

5 Conclusion

Lactic acid bacteria and pathogenic microorganisms accumulate high concentrations of viable cells when cultured alone at a temperature of $37\pm 1^\circ\text{C}$. *Lactobacillus acidophilus* L12 demonstrated the strongest inhibitory activity against the two *Staphylococcus aureus* strains. *Lactobacillus acidophilus* L12 exhibited weaker inhibitory activity against the two *Escherichia coli* strains. *Lactobacillus acidophilus* L12 exhibited the weakest inhibitory activity against the two *Salmonella* sp. strains. The obtained results show the strong antagonistic activity of the potentially probiotic strain *Lactobacillus acidophilus* L12, thus making it a promising candidate for inclusion in the composition of probiotic preparations and functional probiotic foods.

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