KINETICS OF MICROBIAL INACTIVATION OF HUMAN PATHOGENS BY BIOLOGICAL FACTORS

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KEYWORDS

antimicrobial activity, *Lactobacillus*, *Staphylococcus aureus*, *Salmonella abony*, co-culturing, modelling, kinetics

ABSTRACT

The antimicrobial activity of various lactic acid bacteria is an important characteristic for their incorporation in the composition of probiotic preparations and functional foods. The purpose of the present work was to present a mathematical approach to determine the kinetics of antimicrobial action of probiotic lactic acid bacteria *Lactobacillus plantarum* BZ1, *Lactobacillus plantarum* BZ2 and *Lactobacillus plantarum* BZ3 when co-cultured with the pathogenic microorganisms *Staphylococcus aureus* ATCC 25093; *Staphylococcus aureus* ATCC 6538P; *Salmonella* sp. (clinical isolate), *Salmonella abony* NTCC 6017. The pathogen inactivation was achieved by the antagonistic action of the lactic acid bacteria strains, which is a biological factor of inactivation. Three kinetic models to reveal different sides of the antagonism between beneficial lactic acid bacteria and pathogenic microorganisms were used in the present work. Only probiotic strains with good antimicrobial activity against pathogenic microorganisms can be included in the composition of starters for functional foods and beverages and probiotic formulations so that upon consumption the selected lactobacilli strains could execute their inherent role to restore and maintain the microbial balance in the gastrointestinal tract.

INTRODUCTION

A. Theoretical foundations of the kinetics of dying of microorganisms

Mathematically, microbial dying follows one and the same relationship, regardless of the factors that lead to inactivation. Creating inactivation conditions does not lead to the immediate death of the whole cell population. The cells to be destroyed are reduced in number in time under the action of the respective factor. The factors can be chemical, physical and biological. The action of chemical factors (various preservatives and disinfectants) and physical factors (mainly heat generated by various means) is at the heart of sterilization processes in the microbiological industry (Chen et al., 2013).

The biological factors causing a decrease in the number of a group of microorganisms are due to the antagonistic action of beneficial over harmful microorganisms and is expressed in the competitive absorption of the substrate and the production of organic acids, bacteriocins and BLIS and other components causing the inhibitory action against the pathogenic microflora (Denkova et al., 2017).

Microorganisms do not die simultaneously after a certain effect of the inhibitory factor, but by gradually reducing the number of surviving microorganism cells due to their different resistance. If the microbial culture is homogeneous, then the dying rate related to the number of living microorganisms is constant (Stanbury et al., 2003):

$$-\frac{dX}{d\tau} = kX \qquad (1)$$

where: X is the concentration of viable microorganisms (spores of the inactivated microorganism) at the moment τ; k - specific dying rate of the microorganisms, s^{-1}.

Integrating this equation within the limits from N_0 to N and from 0 to τ, the following equation is obtained:

$$\ln\frac{X}{X_0} = -k\tau \qquad (2)$$

where: X_0 - concentration of viable microorganisms to be inactivated in the fermentation volume

This equation in coordinate's ln X-τ is a straight line (Fig. 1). The rate constant is the angular coefficient of the straight line with a negative sign. It is independent of the microorganisms' concentration $\ln X_0$ and the duration of the process and is numerically equal to the proportion of organisms dying per unit time. The physical meaning of the parameter that is inverse of the constant, is the average life span of the individual microorganism during the dying period and characterizes its resistance to the inhibitory factor.

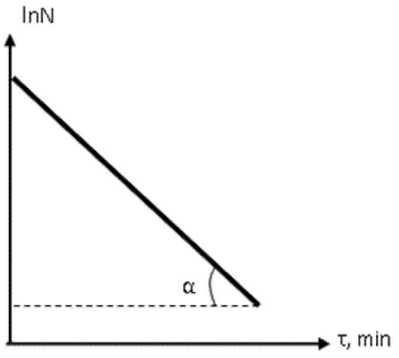

Figure 1: Graph of the kinetics of dying of microorganisms

When studying the influence of various parameters (temperature, pH, etc.) on the destruction of microorganisms, the target function is the rate constant, which characterizes the behavior of microorganisms with average properties, not the number of dead microorganisms.

Communications of the ECMS, Volume 34, Issue 1,
Proceedings, ©ECMS Mike Steglich, Christian Mueller,
Gaby Neumann, Mathias Walther (Editors)
ISBN: 978-3-937436-68-5/978-3-937436-69-2(CD) ISSN 2522-2414

The specific dying rate is a characteristic of the individual microbial species. Physical and chemical effects (Stanbury et al., 2003) have great influence on the constant in addition to the nature of the organism and the conditions for culture growth.

B. Mathematical models for describing the kinetics of dying of pathogenic microorganisms

The following three models were used to model the kinetics of dying of pathogenic microorganisms in the presence of a biological factor:

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

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

$$\frac{dX}{d\tau} = -kX \quad (5)$$

The logistic curve equation (equation 3) describes in general terms the growth of a microbial population in a limited volume. It expresses the effect of the increasing biomass concentration on the maximum specific growth rate. The model has two parameters - the maximum specific growth rate μ and the internal population competition coefficient β. The coefficient β characterizes the effect of the interaction of cells in the microbial population on one another as a result of substrate deficiency and the inhibitory effect of the accumulating metabolic products and shows both the amount of cells killed per unit volume of culture medium per unit time and the inhibition degree of the potential maximum growth rate of the microbial population. This parameter indirectly indicates the influence of the growth conditions on the microbial population. The modified logistic curve model (equation 4) contains the parameter ***n***, which shows the influence of the culture medium composition (local substrate concentrations and metabolic products) on the microbial population. The parameter ***n*** indicates the sensitivity (resistance) of the pathogenic cells to the presence of lactobacilli and the acids and antimicrobial substances produced by the lactobacilli, as well as the sensitivity (resistance) of the lactobacilli cells to the presence of pathogens and their metabolites. Equation (5) is used to describe the kinetics of pathogen cell death. It describes first-order kinetics of chemical reactions. The models presented are generally accepted to describe the kinetics of microbial growth and the inactivation of the microbial population by physical, biological and chemical factors (Denkova et al., 2017; Stanbury et al., 2003)

C. Antimicrobial activity of lactic acid bacteria

Probiotics are „live microorganisms which when administered in adequate amounts confer a health benefit on the host“. Lactic acid bacteria are the major bacterial species used for the production of probiotics and probiotic foods. They are traditional cultures in the production of fermented foods. Probiotic microorganisms contribute to the restoration of the intestinal balance, play an important role in maintaining health and improve the quality of certain foods with their inclusion (Charalampopoulos et al., 2002*;* Charalampopoulos et al.. 2003*;* Stanton et al. 2005*;* Siro et al. 2008; López de Lacey et al. 2014; Soccol et al., 2010; Kociubinski and Salminen, 2006, Denkova-Kostova et al., 2018).

The suppression of conditionally pathogenic, carcinogenic and pathogenic microorganisms is associated with the inactivation of their enzyme systems, inhibition of their adhesion and growth by expelling them from the gastrointestinal tract and normalizing the gastrointestinal microflora. The antimicrobial activity of lactic acid bacteria is mainly related to the production of lactic acid and acetic acid but also to the production of propionic acid, sorbic acid, benzoic acid, hydrogen peroxide, diacetyl, ethanol, phenolic and protein compounds as well as bacteriocins. The produced organic acids alter the medium pH and inhibit the growth of putrefactive, pathogenic and toxigenic microorganisms, while antibacterial substances of peptide nature (bacteriocins) act directly on the microbial cells (Dalié et al., 2010; Eswranandam et al., 2004; Denkova-Kostova et al., 2018).

The purpose of the present work was to study the kinetics of dying of pathogenic microorganisms when co-cultured with lactic acid bacteria. Three mathematical dependencies, which reveal different sides of the process of pathogen inactivation in the presence of the biological factor - the lactic acid bacteria cells, were used to accomplish this purpose.

MATERIAL AND METHODS

A. Microorganisms

- The research was carried out with 3 *Lactobacillus plantarum* strains, isolated from spontaneously fermented vegetables/fruits - *Lactobacillus plantarum* BZ1, *Lactobacillus plantarum* BZ2, *Lactobacillus plantarum* BZ3
- Pathogenic microorganisms: *Staphylococcus aureus* ATCC 25093; *Staphylococcus aureus* ATCC 6538P; *Salmonella* sp. (clinical isolate)*, Salmonella abony* NTCC 6017.

B. Growth media:

- LAPTg10-broth (g/dm^3): peptone - 15; yeast extract - 10; tryptone - 10; glucose - 10. pH was adjusted to 6.6-6.8 and Tween 80 - 1cm^3/dm^3 was added. Sterilization - 20 minutes at 121 °C.
- LAPTg10-agar (g/dm^3): peptone - 15; yeast extract - 10; tryptone - 10; glucose - 10. pH was adjusted to 6.6-6.8 and Tween 80 - 1cm^3/dm^3 and agar - 20 g were added. Sterilization - 20 minutes at 121 °C.
- LBG-agar (g/dm^3): tryptone - 10, yeast extract - 5, NaCl - 10, glucose - 10, agar - 20. Sterilization - 20 minutes at 121 °C.

C. Determination of the antimicrobial activity of *Lactobacillus plantarum* strains against pathogenic microorganisms - by co-cultivation

To determine the antimicrobial activity of the studied *Lactobacillus plantarum* strains against the test-pathogenic microorganisms, the following variants were prepared:

Variant	LAPTg10-broth	*Lactobacillus plantarum*	Pathogen
	cm^3		
LAB C	9.5	0.5	-
Pathogen C	9.5	-	0.5
Mixture	9.0	0.5	0.5

Co-cultivation of each *Lactobacillus plantarum* strain and each pathogen under static conditions in a thermostat at 37±1°C for 60 to 72 hours, taking samples at 0, 12, 24, 36, 48, 60 and 72 h and monitoring the changes in the titratable acidity and the concentration of viable cells of both the pathogen and the *Lactobacillus plantarum* strain, was performed. The number of viable cells was determined through appropriate tenfold dillusions of the samples and spread plating on LBG-agar medium (to determine the number of viable pathogen cells) and on LAPTg10 – agar medium (to determine the number of viable *Lactobacillus plantarum* cells). The Petri dishes were cultured for 72 hours at 37±1°C until the appearance of countable single colonies. The titratable acidity was determined after sterilization of the samples (to kill the pathogen) using 0.1N NaOH. 5 cm^3 of each sample were mixed with 10 cm^3 dH_2O and titrated with 0.1N NaOH, using phenolphtalein as an indicator, until the appearance of pale pink colour, which retained for 1 minute. The value for the titratable acidity was obtained by multiplying the millilitres 0.1N NaOH by the factor of the 0.1N NaOH and the number 20. Bacterial counts were transformed to log values. Results are shown as the average values and standard deviations obtained from three independent experiments (Denkova et al., 2013).

D. Modeling of antimicrobial activity and determination of process kinetic parameters

Equations (3) to (5) have been used to model the process of inactivation of the microbial population of the pathogenic microorganisms. The modeling was performed in an Excel environment, and the accuracy of the models was determined based on the algorithms contained in the software.

RESULTS AND DISCUSSION

Figures 2 to 4 show the growth dynamics of one of the lactobacilli strains (*Lactobacillus plantarum* BZ1) when co-cultured with each of the pathogenic strains. The rest of the figures are of a similar nature and are therefore not presented in the present publication. The results of the identification of the kinetic parameters of the three models are presented in Table 1.

The kinetic parameters presented in the table show that both lactobacilli and pathogens cultured as pure cultures had relatively high maximum specific growth rates. In co-cultivation, the maximum specific growth rates of both lactobacilli and pathogens were reduced. It is noteworthy that the values of the coefficients of internal population competition predicted by the logistic curve model were comparable both in the separate cultivation of the studied strains and in their co-cultivation. The value of β varied from 0.0049 to 0.02833 cfu/cm^3.h. Therefore, for a more detailed study of the growth kinetics of the studied strains and the antimicrobial activity, a modified logistic curve model containing a power factor ***n*** reflecting the effect of the medium composition and the released metabolic products of the tested microorganisms (lactobacilli and pathogens) on the cell growth, was used. The values of the maximum specific growth rates predicted by the two logistic curve models were very close in the separate cultivation of *Lactobacillus plantarum* BZ1, *Lactobacillus plantarum* BZ2 and *Lactobacillus plantarum* BZ3. μ of the strains tested varied in the range of 0.112 to 0.112 h^{-1} according to the classical logistic curve model (model 1); and in the range of 0.100 to 0.103 h^{-1} according to the modified logistic curve model (model 2). Similar values were also observed for the coefficient of internal population competition (β), which, according to models 1 and 2, varied in the range from 0.0078 to 0.0093 cfu/cm^3.h. The parameter ***n*** ranged from 0.8850 to 0.8874. These values indicate that the influence of the medium and the accumulating metabolites (mainly lactic acid) had little effect on the growth of the strains. This was also supported by the high values of the maximum biomass concentration for the three strains predicted by the models (X_k varied from 12.86 to 13.11 log N).

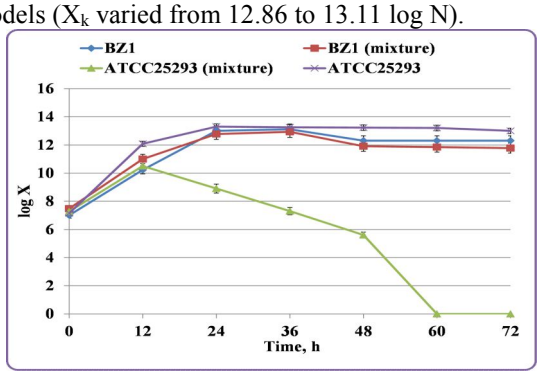

Figure 2: Dynamics of growth of *Lactobacillus plantarum* BZ1 and *Staphylococcus aureus* ATCC 25093 in separate cultivation and in co-cultivation

In separate cultivation, the strains *Staphylococcus aureus* ATCC 25093 and *Staphylococcus aureus* ATCC 6538P were characterized by relatively high values of μ. According to the classical logistic curve model, *Staphylococcus aureus* ATCC 6538P had higher growth rate (0.359 h^{-1}) than *Staphylococcus aureus* ATCC 25093 (μ = 0.144 h^{-1}), whereas, according to Model 2, both strains had close maximum growth rates of 0.105 h^{-1} and 0.104 h^{-1}, respectively. The same trend was observed in the values of the coefficient of internal population competition (0.0078 cfu/cm^3.h for *Staphylococcus aureus* ATCC 6538P and 0.0076 cfu/cm^3.h for *Staphylococcus aureus* ATCC 25093), while higher values of β (0.0283 cfu/cm^3.h and 0.0107 cfu/cm^3.h, respectively) were observed in the classical logistic curve model. Both logistic curve models predicted high concentration of active staphylococci cells varying in the range of 13.30 log N to 13.70 log N. In both *Staphylococcus aureus* strains cultivated individually, the values of the parameter ***n*** were less than 1 (0.8779 and 0.8774, respectively).

Relatively high maximum specific growth rates were also observed in the separate cultivation of *Salmonella*

abony ATCC 6017 and *Salmonella* sp. As a classic model, the logistic curve model predicted higher maximum growth rate for *Salmonella abony* ATCC 6017 (0.295 h^{-1}) than for *Salmonella* sp. (0.119 h^{-1}). A similar trend was observed in the values of β, which was 0.0252 cfu/cm^3.h for *Salmonella abony* ATCC 6017 and 0.0097 cfu/cm^3.h for *Salmonella* sp. According to the modified logistic curve model (model 2), the two pathogens were characterized by close maximum specific growth rates (0.113 h^{-1} for *Salmonella abony* ATCC 6017 and 0.111 h^{-1} for *Salmonella* sp.). The same trend was observed for the values of the parameter β (0.0095 cfu/cm^3.h and 0.0092 cfu/cm^3.h, respectively).
For *Salmonella* sp. both logistic curve models predicted higher maximum concentrations of active cells (12.21 log N by the classical logistic curve model and 12.10 log N by the modified logistic curve model) compared to *Salmonella abony* ATCC 6017 (11.70 log N and 11.82 log N, respectively). The co-cultivation of *Lactobacillus plantarum* BZ1 and *Staphylococcus aureus* ATCC 25093 (Figure 1), *Staphylococcus aureus* ATCC 6538P, *Salmonella abony* ATCC 6017 and *Salmonella* sp. showed a slight decrease in the maximum specific growth rate of *Lactobacillus plantarum* BZ1, which according to the two logistic curve models ranged from 0.074 h^{-1} to 0.098 h^{-1} (μ ranged between 0.101 h^{-1} and 0.121 h^{-1} in separate cultivation). Comparable values were also observed for β, which varied in the range of 0.0022 cfu/cm^3.h to 0.0077 cfu/cm^3.h.

Table 1: Kinetic characteristics of pathogen inactivation upon co-cultivation with *Lactobacillus plantarum* strains

Variant	Kinetic parameters							
	Equation 3			Equation 4				Equation 5
	μ	β	$X_к$	μ	β,	$X_к$	n	k,
	h^{-1}	cfu/cm^3.h	cfu/cm^3	h^{-1}	cfu/cm^3.h	cfu/cm^3		h^{-1}
L.plantarum BZ1 control	0.121	0.0093	13.11	0.101	0.0078	13.00	0.8850	-
L.plantarum BZ2 control	0.114	0.0088	12.94	0.100	0.0078	12.86	0.8866	-
L.plantarum BZ3 control	0.112	0.0085	13.07	0.103	0.0079	12.95	0.8874	-
St. aureus ATCC 25093 control	0.144	0.0107	13.52	0.104	0.0076	13.70	0.8779	-
St. aureus ATCC 6538P control	0.359	0.0283	13.30	0.105	0.0078	13.35	0.8774	-
S. abony ATCC 6017 control	0.295	0.0252	11.70	0.113	0.0095	11.82	0.8255	-
Salmonella sp. control	0.119	0.0097	12.21	0.111	0.0092	12.10	0.8841	-
***L. plantarum* BZ1+*St. aureus* ATCC 25093**								
L.plantarum BZ1 (in mixture)	0.087	0.0060	12.95	0.074	0.0057	12.92	0.9056	-
St. aureus ATCC 25093 (in mixture)	0.078	0.0067	11.55	0.119	0.0111	10.43	1.1232	0.313
***L. plantarum* BZ2 + *St. aureus* ATCC 25093**								
L.plantarum BZ2 (in mixture)	0.092	0.0070	12.78	0.084	0.0066	12.75	0.9490	-
St. aureus ATCC 25093 (in mixture)	0.088	0.0080	11.04	0.086	0.0065	13.20	1.1948	0.318
***L. plantarum* BZ3 + *St. aureus* ATCC 25093**								
L.plantarum BZ3 (in mixture)	0.090	0.0071	12.72	0.084	0.0066	12.66	0.9538	-
St. aureus ATCC 25093 (in mixture)	0.081	0.0072	11.31	0.097	0.0066	10.51	1.2000	0.325
***L.plantarum* BZ1 + *St. aureus* ATCC 6538 P**								
L.plantarum BZ1 (in mixture)	0.092	0.0072	12.78	0.080	0.0066	12.74	0.9137	-
St. aureus ATCC 6538 P (in mixture)	0.063	0.0049	12.80	0.022	0.0019	10.41	1.3523	0.317
***L.plantarum* BZ2 + *St. aureus* ATCC 6538 P**								
L.plantarum BZ2 (in mixture)	0.087	0.0067	12.96	0.085	0.0065	12.91	0.9932	-
St. aureus ATCC 6538 P (in mixture)	0.074	0.0065	11.56	0.022	0.0021	10.48	1.5623	0.307
***L.plantarum* BZ3 + *St. aureus* ATCC 6538 P**								
L.plantarum BZ3 (in mixture)	0.079	0.0062	12.87	0.084	0.0067	12.52	0.9069	-
St. aureus ATCC 6538 P (in mixture)	0.077	0.0065	11.83	0.019	0.0019	10.50	1.7424	0.307
***L.plantarum* BZ1 + *S. abony* ATCC 6017**								
L.plantarum BZ1 (in mixture)	0.082	0.0060	13.29	0.081	0.0022	13.30	0.9075	-
S. abony ATCC 6017 (in mixture)	0.107	0.0090	11.84	0.115	0.0102	11.27	0.9032	0.449
***L.plantarum* BZ2 + *S. abony* ATCC 6017**								
L.plantarum BZ2 (in mixture)	0.099	0.0076	13.02	0.098	0.0022	12.84	0.9088	-
S. abony ATCC 6017 (in mixture)	0.099	0.0084	11.70	0.115	0.0101	11.05	0.9976	0.462
***L.plantarum* BZ3 + *S. abony* ATCC 6017**								
L.plantarum BZ3 (in mixture)	0.080	0.0060	13.30	0.081	0.0022	13.50	0.9054	-
S. abony ATCC 6017 (in mixture)	0.098	0.0085	11.55	0.098	0.0086	11.43	0.9753	0.462
***L.plantarum* BZ1 + *Salmonella* sp.**								
L.plantarum BZ1 (in mixture)	0.095	0.0077	12.95	0.098	0.0077	13.17	0.8909	-
Salmonella sp. (in mixture)	-	-	-	-	-	-	-	0.587
***L.plantarum* BZ2 + *Salmonella* sp.**								
L.plantarum BZ2 (in mixture)	0.089	0.0068	13.15	0.076	0.0057	13.23	1.1179	-
Salmonella sp. (in mixture)	-	-	-	-	-	-	-	0.628
***L.plantarum* BZ3 + *Salmonella* sp.**								
L.plantarum BZ3 (in mixture)	0.089	0.0069	12.89	0.076	0.0056	13.56	1.0928	-
Salmonella sp. (in mixture)	-	-	-	-	-	-	-	0.394

A slight increase in the parameter ***n***, which varied in the range from 0.8909 to 0.9137, were observed in the co-cultivation of *Lactobacillus plantarum* BZ1 and the pathogens studied. This indicates that *Lactobacillus plantarum* BZ1 was very slightly affected by the presence of the pathogens and the metabolites secreted during their growth. The high values of the maximum active cell concentration of *Lactobacillus plantarum*

BZ1 predicted by both models also serve as a confirmation of this conclusion. The value for X_k was close to that of the control (separate cultivation of the strain) - from 12.74 log N to 13.30 log N. In the co-cultivation of *Staphylococcus aureus* ATCC 25093 or *Staphylococcus aureus* ATCC 6538P and *Lactobacillus plantarum* BZ1, a reduction in the maximum specific growth rate of the pathogens, especially for *Staphylococcus aureus* ATCC 6538P, in which μ decreased from 0.359 h^{-1} to 0.063 h^{-1}, according to the classical logistic curve model, and to 0.019 h^{-1}, according to model 2, was observed. According to both models for this strain, β ranged from 0.0019 cfu/cm^3.h to 0.0077cfu/cm^3.h.

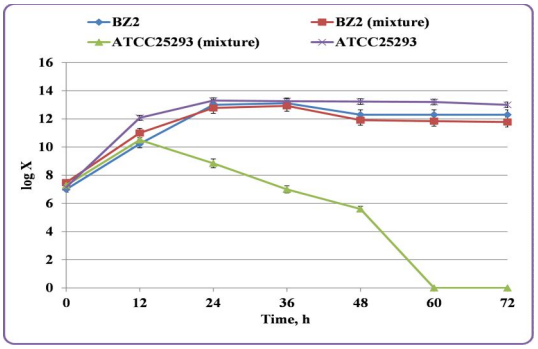

Figure 3: Dynamics of growth of *Lactobacillus plantarum* BZ2 and *Staphylococcus aureus* ATCC 25093 in separate cultivation and in co-cultivation

In *Staphylococcus aureus* ATCC 25093, a reduction in the maximum specific growth rate to 0.078 h^{-1} and 0.119 h^{-1} and in the internal population competition β to 0.0067 cfu/cm^3.h and 0.0111 cfu/cm^3.h, according to the two logistic curve models used was observed. What is striking are the high values of the parameter ***n***, which was 1.1232 for *Staphylococcus aureus* ATCC 25093 and 1.3523 for *Staphylococcus aureus* ATCC 6538P. This indicated that the pathogenic microorganisms were strongly influenced by the presence of the lactobacilli and the released substances with antimicrobial activity (organic acids, bacteriocins, etc.). This was also confirmed by the fact that, according to the mathematical models, both pathogens were characterized by a significantly lower maximum concentration of active pathogen cells in the mixed population, which varied in the range from 10.41 log N to 12.80 log N for *Staphylococcus aureus* ATCC 6538P and from 10.43 log N to 11.55 log N for *Staphylococcus aureus* ATCC 25093. In the separate cultivation both pathogens showed a maximum final concentration of active cells in the range from 13.52 log N to 13.70 log N for *Staphylococcus aureus* ATCC 25093 and from 13.30 log N to 13.35 log N for *Staphylococcus aureus* ATCC 6538P. Comparable values of the dying rate constant were observed in the conducted modelling of the kinetics of dying of the pathogenic strains of *Staphylococcus aureus* - 0.313 h^{-1} for *Staphylococcus aureus* ATCC 25093 and 0.317 h^{-1} for *Staphylococcus aureus* ATCC 6538P.

The co-cultivation of *Salmonella abony* ATCC 6017 and *Lactobacillus plantarum* BZ1 resulted in a reduction in the maximum specific growth rate of the pathogen, but to a lesser extent than that of *Staphylococcus aureus*. In this strain, μ varied in the range from 0.107 h^{-1} and 0.115 h^{-1}, with the parameter β varying from 0.0090 cfu/cm^3.h to 0.0102 cfu/cm^3.h. A lower value of the parameter ***n*** (n=0.9032) was also observed in this strain compared to the two representatives of *Staphylococcus aureus*. This indicated that *Salmonella abony* ATCC 6017 would exhibit resistance to the presence of lactobacilli and their metabolites in comparison with the two strains of *Staphylococcus aureus*. This was further confirmed by the fact that the values of the maximum active cell concentration of *Salmonella abony* ATCC 6017 in the mixed population were commensurable with that of the control (pathogen separate cultivation), namely 11.84 log N and 11.27 log N. However, the rate constant of dying of the pathogen was 0.449 h^{-1} and it was higher than that of *Staphylococcus aureus*.

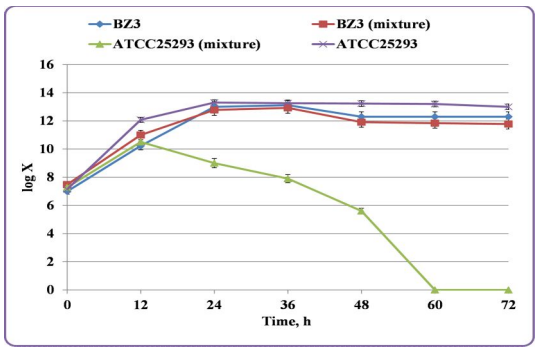

Figure 4: Dynamics of growth of *Lactobacillus plantarum* BZ3 and *Staphylococcus aureus* ATCC 25093 in separate cultivation and in co-cultivation

The co-cultivation of *Salmonella* sp. and *Lactobacillus plantarum* BZ1 resulted in a complete reduction of the maximum specific growth rate compared to that in the separate cultivation of the pathogen alone. Since the beginning of co-cultivation, there had been continuous death of the pathogen cells. The rate constant of dying was 0.587 h^{-1} in the co-cultivation of *Salmonella* sp. and *Lactobacillus plantarum* BZ1 and it was the highest compared to that of the other pathogenic microorganisms.

The co-cultivation of *Lactobacillus plantarum* BZ2 (Figure 3) with the pathogens examined showed a similar trend as in the previous strain *Lactobacillus plantarum* BZ1. A slight reduction in the maximum specific growth rate was observed, which varied from 0.087 h^{-1} to 0.099 h^{-1}, and β ranged from 0.0067 cfu/cm^3.h to 0.0076 cfu/cm^3.h, according to model 1 and, μ varied from 0.076 h^{-1} to 0.098 h^{-1}, and β ranged from 0.0022 cfu/cm^3.h to 0.0066 cfu/cm^3.h, according to model 2. Again, a slight increase in the parameter ***n*** was observed in this strain, whose values ranged from 0.9088 to 1.1179. This indicated that this strain was also poorly affected by the presence of the studied pathogens and their metabolites. As a confirmation of this conclusion was the high value of the maximum concentration of active cells - X_k varied in the range from 12.78 log N to 13.15 log N according to model 1

and from 12.75 log N to 13.23 log N according to model 2 and these values were close to those of the control.

The co-cultivation of *Staphylococcus aureus* ATCC 25093 or *Staphylococcus aureus* ATCC 6538P and *Lactobacillus plantarum* BZ2 resulted in a reduction in the pathogen maximum specific growth rate. μ for *Staphylococcus aureus* ATCC 25093 changed from 0.086 h^{-1} to 0088 h^{-1}, and β ranged from 0.0065 $cfu/cm^3.h$ to 0.0080 $cfu/cm^3.h$, according to the mathematical models used. A maximum reduction in the maximum specific growth rate of *Staphylococcus aureus* ATCC 6538P - between 0.022 h^{-1} and 0.074 h-1 was observed, while β varied between 0.0021 $cfu/cm^3.h$ and 0.0065 $cfu/cm^3.h$. The parameter ***n*** had a higher value (n=1.5623) in the co-cultivation of *Staphylococcus aureus* ATCC 6538P and *Lactobacillus plantarum* BZ2 compared to *Staphylococcus aureus* ATCC 25093 (n=1.1948). This indicated that *Staphylococcus aureus* ATCC 6538P was more strongly influenced by the presence of *Lactobacillus plantarum* BZ2 and the secreted metabolites with antimicrobial activity, which was also evidenced by the lower maximum growth rates of this strain in the mixed population. The higher sensitivity of this pathogen to lactic acid bacteria was also confirmed by the lower values of the maximum concentration of active cells in the mixed population for *Staphylococcus aureus* ATCC 6538P, which, according to the models, varies from 10.48 log N and 11.56 log N, compared with that of *Staphylococcus aureus* ATCC 25093, which, according to the mathematical models, varied in the range from 11.04 log N to 13.20 log N.

Staphylococcus aureus ATCC 25093 or *Staphylococcus aureus* ATCC 6538P cultured in a mixed population with *Lactobacillus plantarum* BZ2 were characterized by compatible and relatively high dying rates - 0.318 h^{-1} for *Staphylococcus aureus* ATCC and 0.307 h^{-1} for *Staphylococcus aureus* ATCC 6538P.

The co-cultivation of *Salmonella abony* ATCC 6017 and *Lactobacillus plantarum* BZ2 resulted in a reduction in the maximum specific growth rate of the pathogen, once again to a lesser extent than that of the two *Staphylococcus aureus* strains. In the co-cultivation of *Salmonella abony* ATCC 6017 and *Lactobacillus plantarum* BZ2, μ for *Salmonella abony* ATCC 6017 changed between 0.099 h^{-1} and 0.115 h^{-1}, and β ranged from 0.0084 $cfu/cm^3.h$ to 0.0101 $cfu/cm^3.h$. The parameter ***n*** value was lower (n=0.9776) compared to the same parameter in the co-cultivation of *Lactobacillus plantarum* BZ2 with the two representatives of *Staphylococcus aureus*, indicating resistance of the pathogen to the presence of *Lactobacillus plantarum* BZ2 and its metabolic products. To support this, the maximum concentration of pathogen active cells in the mixed population was 11.05 log N and 11.70 log N, which was close to that of the control (separate cultivation of *Salmonella abony* ATCC 6017) - 11.70 log N and 11.82 log N. Nevertheless, the dying rate of *Salmonella abony* ATCC 6017 was significantly higher than that of *Staphylococcus aureus* ATCC 25093 and *Staphylococcus aureus* ATCC 6538P. For *Salmonella abony* ATCC 6017, the dying constant was 0.462 h^{-1}.

A complete reduction of the maximum specific growth rate in comparison with the separate cultivation of *Salmonella* sp. alone was observed in the co-cultivation of *Salmonella* sp. and *Lactobacillus plantarum* BZ2. From the beginning of the co-cultivation, there had been determined continuous death of the pathogen cells. In the co-cultivation of *Salmonella* sp. and *Lactobacillus* plantarum BZ2, the dying rate constant for *Salmonella* sp. was the highest (0.628 h^{-1}) compared to the same parameter for the other pathogens. This value of the dying rate constant was higher but close to the dying rate constant value of *Salmonella* sp. in the co-cultivation of *Salmonella* sp. and *Lactobacillus plantarum* BZ1 (0.587 h^{-1}). This in turn indicated that *Salmonella* sp. was more sensitive to the presence of *Lactobacillus plantarum* BZ2 and its metabolites secreted in the medium.

In co-cultivation of *Lactobacillus plantarum* BZ3 with the pathogens examined, a slight reduction in the maximum specific growth rate of *Lactobacillus plantarum* BZ3 was observed, varying from 0.076 h^{-1} to 0.089 h^{-1}, and β ranging from 0.0060 $cfu/cm^3.h$ to 0.0071 $cfu/cm^3.h$ according to model 1; μ varied from 0.081 h^{-1} to 0.084 h^{-1}, and β ranged from 0.0022 $cfu/cm^3.h$ to 0.0066 $cfu/cm^3.h$ according to model 2. Once again, a slight increase in the parameter ***n*** for *Staphylococcus aureus* ATCC 25093, *Staphylococcus aureus* ATCC 6538P and *Salmonella abony* ATCC 6017 was observed. Its values were 0.9538, 0.9069 and 0.9054, respectively, which indicated that *Lactobacillus plantarum* BZ3 was also affected by the presence of these pathogenic strains and their metabolites. This was evidenced by the high values of the maximum concentration of active lactobacilli cells in the mixed population, which varied for the respective pathogens – between 12.72 log N and 12.66 log N in the co-cultivation with *Staphylococcus aureus* ATCC 25093; between 12.87 log N and 12.52 log N in the co-cultivation with *Staphylococcus aureus* ATCC 6538P; between 13.30 log N and 13.50 log N in the co-cultivation with *Salmonella abony* ATCC 6017. These values weare close to those of the control (*Lactobacillus plantarum* BZ3 cultivated alone). The co-cultivation of *Lactobacillus plantarum* BZ3 and *Salmonella* sp. resulted in a higher value of ***n*** (n=1.0918), compared to the other *Lactobacillus plantarum* strains tested. However, *Lactobacillus plantarum* BZ3 also achieved high maximum final concentration of active cells in the mixed population of 12.89 log N and 13.56 log N, indicating a negligible effect of the pathogen and its metabolites on the lactobacilli cells.

In the co-cultivation of *Staphylococcus aureus* ATCC 25093 or *Staphylococcus aureus* ATCC 6538P and *Lactobacillus plantarum* BZ3, a reduction in the maximum specific growth rate of the pathogens was observed. For *Staphylococcus aureus* ATCC 25093 μ varied from 0.081 h^{-1} to 0.097 h^{-1}, and β ranged from

0.0066 cfu/cm^3.h to 0.0072 cfu/cm^3.h, according to the mathematical models used. Again, *Staphylococcus aureus* ATCC 6538P showed greater reduction in the maximum specific growth rate - between 0.019 h^{-1} and 0.077 h^{-1}, while β varied between 0.0019 cfu/cm^3.h and 0.0065 cfu/cm^3.h. In the co-cultivation of *Staphylococcus aureus* ATCC 6538P and *Lactobacillus plantarum* BZ3, the parameter ***n*** had higher value (n=1.7424) compared to the co-cultivation of the same lactobaciili strain and *Staphylococcus aureus* ATCC 25093 (n=1.2000). The value of ***n*** in *Staphylococcus aureus* ATCC 6538P was the highest compared to the values in co-cultivation of the same pathogenic strain with the other lactobacilli strains, indicating that this pathogen was most sensitive to the presence of *Lactobacillus plantarum* BZ3, compared to the other *Lactobacillus plantarum* strains. The same trend was observed for *Staphylococcus aureus* ATCC 25093. The high impact of *Lactobacillus plantarum* BZ3 on these two pathogens can also be seen in the significantly lower values of the maximum final concentrations of active pathogen cells in the mixed populations, compared to the controls. The maximum final active cell concentration varied from 10.51 log N to 11.31 log N for *Staphylococcus aureus* ATCC 25093 and from 11.38 log N to 10.50 log N for *Staphylococcus aureus* ATCC 6538P.

Staphylococcus aureus ATCC 25093 and *Staphylococcus aureus* ATCC 6538P co-cultured with *Lactobacillus plantarum* BZ3 were again characterized by consistent and relatively high dying rates - 0.325 h^{-1} for *Staphylococcus aureus* ATCC 25093 and 0.307 h^{-1} for *Staphylococcus aureus* ATCC 6538P.

In the co-cultivation of *Salmonella abony* ATCC 6017 and *Lactobacillus plantarum* BZ3, a reduction in the maximum specific growth rate of the pathogen was observed, with both models predicting an equal reduction in the maximum specific growth rate to 0.098 h^{-1}, as well as close β values of 0.0085 cfu/cm^3.h and 0.0086 cfu/cm^3.h. The parameter ***n*** (0.9753) was lower in the co-cultivation of *Salmonella abony* ATCC 6017 and *Lactobacillus plantarum* BZ3 than in the co-culturing of the same lactobacilli strain and the representatives of *Staphylococcus aureus*, indicating resistance of *Salmonella abony* ATCC 6017 to the presence of *Lactobacillus plantarum* BZ3 and its metabolites. The maximum concentration of pathogen active cells in the mixed population can serve as as evidence - 11.43 log N and 11.55 log N, which was close to the values of the control (separate cultivation of *Salmonella abony* ATCC 6017 alone) - 11.70 log N and 11.82 log N .

Salmonella abony ATCC 6017 dying rate in the co-culturing of *Salmonella abony* ATCC 6017 and *Lactobacillus plantarum* BZ3 was significantly higher than that of *Staphylococcus aureus* ATCC 25093 and *Staphylococcus aureus* ATCC 6538P. The *Salmonella abony* ATCC 6017 dying rate value was equal to that in the co-cultivation of the same pathogen with *Lactobacillus plantarum* BZ2 - 0.462 h^{-1}.

A complete reduction of the maximum specific growth rate of *Salmonella* sp. in the co-cultivation of *Salmonella* sp. and *Lactobacillus plantarum* BZ3 compared to the cultivation of the pathogen alone was observed. From the beginning of the co-cultivation, there had been continuous dying of the pathogen cells. In the co-cultivation of *Salmonella* sp. and *Lactobacillus plantarum* BZ3, a lower value of the dying rate constant (0.394 h^{-1}) compared to the co-cultivation of this pathogen with the other lactobacilli strains was observed. This lower value of the duing rate constant indicated that *Salmonella* sp. was resistant to the presence of *Lactobacillus plantarum* BZ3 and its metabolites.

The models used had high accuracy, ranging from 0.85 to 0.99 (evaluated by the R^2-value). They were distinguished by their simple and high appreciation of the inactivation of the pathogens. The data in Table 1 show that the three strains tested had similar values with respect to the kinetic parameters of pathogen inactivation. This was due to the fact that they had been isolated from similar sources, suggesting similarities in their specific metabolism, including the principles and mechanisms of inactivation of pathogenic microorganisms.

CONCLUSION

The antimicrobial activity of lactic acid bacteria against pathogens is a paramount prerequisite for their selection for inclusion in the composition of probiotic preparations and different functional foods. The kinetics of the antimicrobial activity of three *Lactobacillus plantarum* strains against 2 *Staphylococcus aureus* strains and 2 *Salmonella* strains was determined using 3 kinetic models. The classical logistic curve equation and the modified logistic curve equation revealed different sides of the antagonism between beneficial *Lactobacillus plantarum* strains and the pathogenic microorganisms and the very inactivation of the pathogens under the action of this biological factor. The kinetic parameters showed that *Salmonella* sp. was the most sensitive pathogen to the presence of the *Lactobacillus plantarum* strains and their metabolites, followed by *Staphylococcus aureus* ATCC 6538P, *Staphylococcus aureus* ATCC 25093 and *Salmonella abony* ATCC 6017. The applied kinetic models were adequate and appropriate for examination of the antagonism kinetics between the lactic acid bacteria strains and the pathogen strains.

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ACKNOWLEDGEMENTS

This work were supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM # 577/17.08.2018 and by the Bulgarian National Fund "Scientific Research" under the project КП-06-Rila/2 from 20.12.2018 "Bio-pr**E**servation by the **S**ynergisti**C** Action of **P**robiotics and plant **E**xtracts (ESCAPE)". Cooperation between University of Food Technologies Plovdiv (Bulgaria), BioDyMIA research unit (EA n°3733, Université Claude Bernard Lyon 1 - ISARA Lyon, France), and PAM (Université de Bourgogne - AgroSup Dijon Joined Research Unit) was supported by Franco-Bulgarian cooperation Hubert Curien Programme (PHC Rila) (ESCAPE project). The authors wish to express their gratitude for the supports of the Ministry of Education and Science (Bulgaria), the Embassy of France to Bulgaria in Sofia ("Ministère des Affaires Etrangères", France), Campus France and "Ministère de l'Enseignement Supérieur, de la Recherche et de l'Innovation" (France).

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