Antimicrobial activity of *Lactobacillus delbrueckii* ssp. *bulgaricus* strains against *Candida albicans* NBIMCC 74

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Abstract: *The antimicrobial activity of Lactobacillus delbrueckii ssp. bulgaricus B1 and Lactobacillus delbrueckii ssp. bulgaricus TAB2 against Candida albicans NBIMCC 74 in co-culture and at single strain culture at a temperature of 37±1°C was examined. During co-cultivation an increase in the concentration of viable lactobacilli cells by the 24th hour was established and it reached above 1.10^{11}cfu/cm^3, and then remained relatively constant. The number of viable cells of Candida albicans NBIMCC 74 in co-cultivation with each of the two Lactobacillus delbrueckii ssp. bulgaricus strains, was greatly reduced and by the 72nd hour it reached 1.10^{5}cfu/cm^3. The observed antimicrobial activity was due to a great extent to the acidification of the medium because of the production and accumulation of lactic acid. The demonstrated antimicrobial activity is a prerequisite for further research on the probiotic potential of the two Lactobacillus delbrueckii ssp. bulgaricus strains for their inclusion in the composition of probiotic preparations.*

Key words: Probiotic, *Lactobacillus delbrueckii* ssp. *bulgaricus*, Antimicrobial, Co-cultivation, *Candida albicans*

INTRODUCTION

Lactic acid bacteria are Gram-positive, nonmotile, nonsporeforming and rod- and coccus-shaped organisms that can ferment carbohydrates mainly producing lactic acid. Probiotic lactic acid bacteria show attractive therapeutic properties and technological applications, such as proteolytic activity; lactose and citrate fermentation; production of polysaccharides; high resistance to freezing and freeze-drying; capacity for adhesion and colonization in digestive mucosa; production of vitamins; and production of antimicrobial compounds [1, 5, 7].

The probiotic lactic acid bacteria could be present in the spontaneous fermentation of different foods [4]. They have been used as starter cultures, and they have become widespread in the manufacture of fermented vegetables and dairy and meat products [3, 6].

The purpose of the present work was to study the antimicrobial activity of *Lactobacillus delbrueckii* ssp. *bulgaricus* B1 and *Lactobacillus delbrueckii* ssp. *bulgaricus* TAB2 against *Candida albicans* NBIMCC 74.

MATERIALS AND METHODS

Microorganisms

Lactobacillus delbrueckii ssp. *bulgaricus* B1 and *Lactobacillus delbrueckii* ssp. *bulgaricus* TAB2, isolated from salad dressings;

test microorganism *Candida albicans* NBIMCC 74

Media

- MRS – broth medium

Composition (g/dm^3): 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 cm^3/dm^3; pH = 6.5. Sterilization - 15 minutes at 118ºC.

- LAPTg10 – agar medium

Composition (g/dm^3): peptone - 15; yeast extract - 10; tryptone - 10; glucose – 10; Tween 80-1 cm^3/dm^3, agar - 15. pH=6.6 - 6.8. Sterilization - 20 minutes at 121ºC.

- LBG – agar medium

Composition (g/dm^3): tryptone - 10; yeast extract - 5; NaCl - 10; glucose - 10; agar - 15; pH = 7,5. Sterilization - 20 minutes at 121°C.

Determination of the antimicrobial activity against pathogenic microorganisms - by co-culturing

To determine the antimicrobial activity of the studied strains of lactobacilli against

Candida albicans NBIMCC 74 a 48 hour cultural suspension of each *Lactobacillus delbrueckii* ssp. *bulgaricus* strain was used. Separate cultivation of the two *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Candida albicans* NBIMCC 74 strain as well as co-cultivation of each of the two *Lactobacillus delbrueckii* ssp. *bulgaricus* strains with *Candida albicans* NBIMCC 74 included in the study were conducted. For the examination of the co-cultivation 0.5 cm^3 of the suspension of the *Lactobacillus delbrueckii* ssp. *bulgaricus* strain, 0.5 cm^3 of the suspension of *Candida albicans* NBIMCC 74 strain and 9 cm^3 of culture medium (MRS-broth medium) were mixed. In the control of each *Lactobacillus delbrueckii* ssp. *bulgaricus* and in the control of *Candida albicans* NBIMCC 74 9.5 cm^3 of the MRS-broth medium were mixed with 0.5 cm3 of the suspension of the *Lactobacillus delbrueckii* ssp. *bulgaricus* strain or of the suspension of *Candida albicans* NBIMCC 74 strain, respectively, under static conditions in a thermostat at 37±1ºC for 72 hours, taking samples at 0, 12, 24, 36, 48, 60 and 72 h and monitoring the change of the titratable acidity and the concentration of viable cells of both the pathogen and the *Lactobacillus delbrueckii* ssp. *bulgaricus* strains was performed. Determination of the number of viable cells was done by the spread plate method on LAPTg10-agar (for the enumeration of lactobacilli), on LBG-agar (for the enumeration of pathogen). The titratable acidity was determined according to a standard protocol [2].

Modeling of kinetics

For the modeling of the kinetics of growth of the *Lactobacillus delbrueckii* ssp. *bulgaricus* strains and the pathogen the logistic curve equation (Verhulst equation) was used. The kinetics of dying were defined according to the exponential equation:

$$\left|\begin{array}{l} \dfrac{dX}{d\tau} = [\mu_m - \beta X\,]X \Rightarrow X = \dfrac{X_н e^{\mu_m(\tau-\tau_{lag})}}{1 - \dfrac{X_н}{X_{кр}}\left(1 - e^{\mu_m(\tau-\tau_{lag})}\right)} \\ \dfrac{dX}{d\tau} = -kX \Rightarrow X = X_н e^{-kt} \end{array}\right.$$

Wherein: $X_н$ и $X_{кр}$ are the starting concentration and the final concentration of cells, cfu/cm^3; μ_m is the maximum specific growth rate, h^{-1}; β - coefficient of internal competition population, cfu/cm^3.h; k – dying rate, s^{-1}; τ_{log} – duration of the lag-phase, h; τ - time, h.

The kinetic parameters in the model were determined by analogy with [Kostov, 2015], after linearization of the equation under the condition that Δt = const:

$$\psi = 1 - \frac{X_t}{X_{t+\Delta t}} = 1 - \left(1 - \frac{X}{X_K}\right)\exp(-\mu_m \Delta t)$$

RESULTS AND DISCUSSION

In a study of the antimicrobial activity of lactic acid bacteria against pathogenic microorganisms by the method of co-cultivation it is important to identify the specific growth rates of both the *Lactobacillus* strain and the pathogen. Thus the dynamics of the change in the number of viable cells and in the titratable acidity were monitored (Fig. 1, Fig. 2, Fig. 3, Fig. 4).

In the co-cultivation of both *Lactobacillus delbrueckii* ssp. *bulgaricus* strains and *Candida albicans* NBIMCC 74 strain, the *Lactobacillus* strains were not significantly influenced by the presence of *Candida albicans* NBIMCC 74. But the number of viable cells of the pathogens was greatly reduced in a strain-specific manner.

In single-strain cultivation of the two *Lactobacillus delbrueckii* ssp. *bulgaricus* strains high concentrations of viable cells were achieved by the 24th hour and they were maintained by the end of the cultivation. In single-strain cultivation of *Candida albicans*

NBIMCC 74 high concentration of viable cells was achieved by the 48 - 72nd hour. In co-cultivation inhibition of the growth of *Candida albicans* NBIMCC 74 was observed, the reduction of the concentration of viable cells of the pathogen being greater in co-cultivation with *Lactobacillus delbrueckii* ssp. *bulgaricus* TAB2. The number of viable cells of *Candida albicans* NBIMCC 74 in co-cultivation with both *Lactobacillus delbrueckii* ssp. *bulgaricus* strains reached 1.10^5cfu/cm^3 at the end of the process (Fig. 1, Fig. 3). The observed antimicrobial activity was due to a great extent to the acidification of the medium because of the production and accumulation of lactic acid. (Fig. 2, Fig. 4).

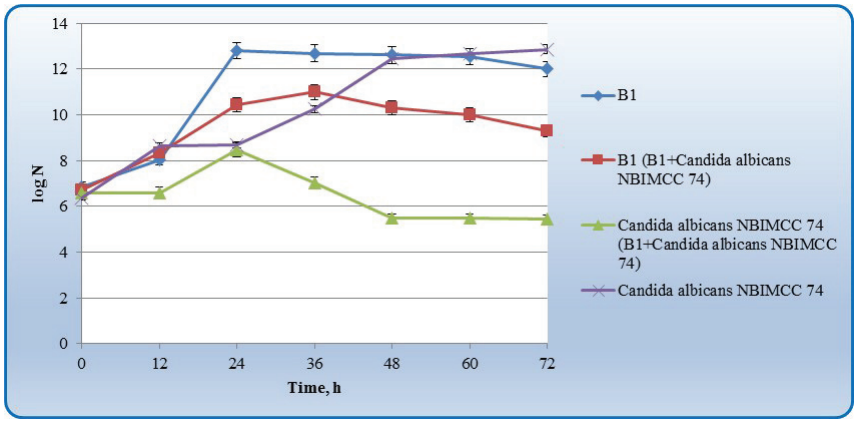

Fig. 1. Survival of *Lactobacillus delbrueckii* ssp. *bulgaricus* B1 and *Candida albicans* NBIMCC 74 in single-strain culture and in a mixed population at 37±1°C

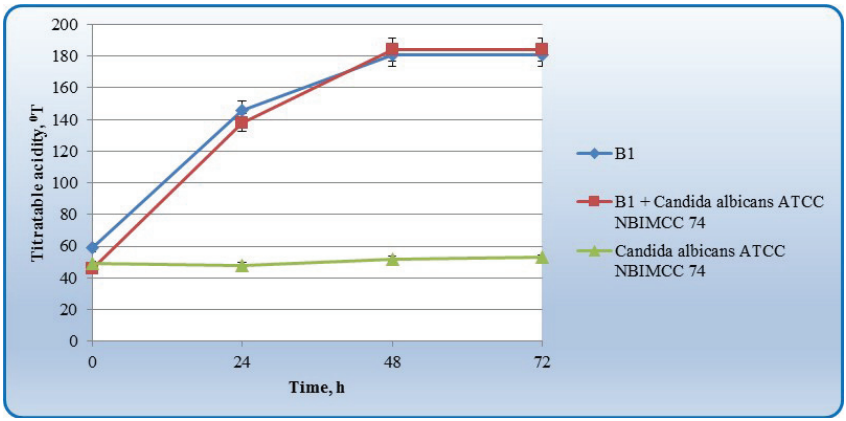

Fig. 2. Changes in the titratable acidity of the medium in single-strain culture and in a mixed population of *Lactobacillus delbrueckii* ssp. *bulgaricus* B1 and *Candida albicans* NBIMCC 74 at 37±1°C

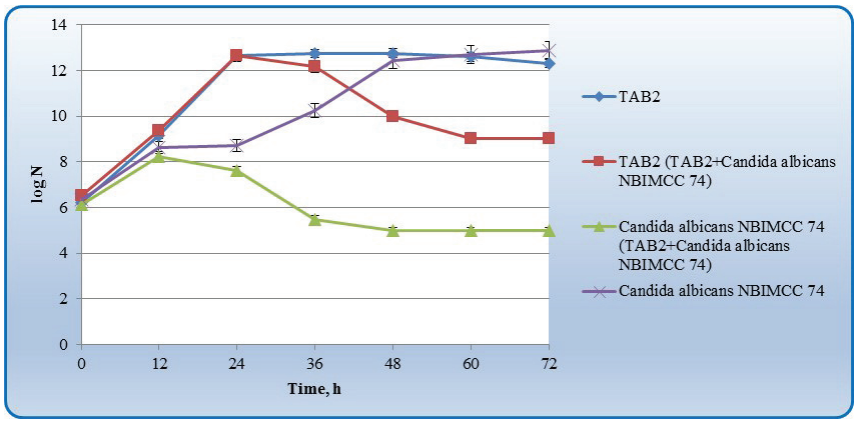

Fig. 3. Survival of *Lactobacillus delbrueckii* ssp. *bulgaricus* TAB2 and *Candida albicans* NBIMCC 74 in single-strain culture and in a mixed population at 37±1°C

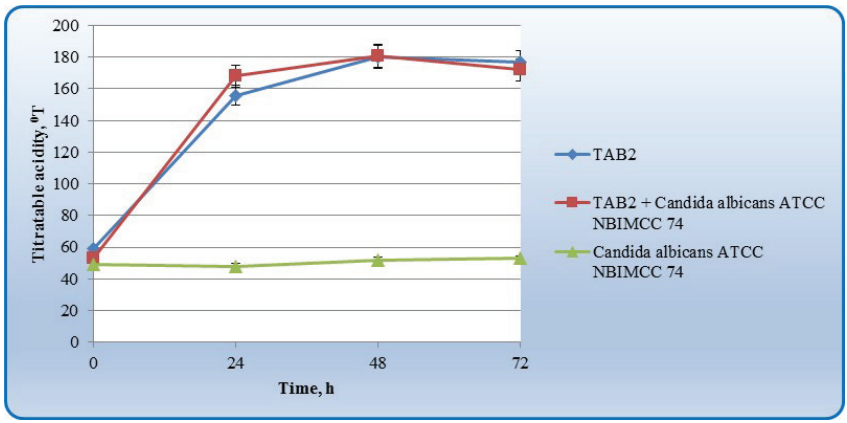

Fig. 4. Changes in the titratable acidity of the medium in single-strain culture and in a mixed population of *Lactobacillus delbrueckii* ssp. *bulgaricus* TAB2 and *Candida albicans* NBIMCC 74 at 37±1°C

The maximum specific growth rates of the two *Lactobacillus delbrueckii* ssp. *bulgaricus* strains and *Candida albicans* NBIMCC 74 as single-strain cultures and in a mixed population were calculated by the equation of the logistic curve (Table 1).

In the co-cultivation of *Candida albicans* NBIMCC 74 and *Lactobacillus delbrueckii* ssp. *bulgaricus* TAB2 high maximum specific growth rate (0,105 h^{-1}) was observed, which was the cause of the increase in the concentration of the pathogen cells in the beginning of the process. In the co-cultivation of *Candida albicans* NBIMCC 74 and *Lactobacillus delbrueckii* ssp. *bulgaricus* B1 was observed lower growth rate (0,06 h^{-1}) was established. The coefficient of internal population competition of *Candida albicans* NBIMCC 74 is higher in co-cultivation with *Lactobacillus delbrueckii* ssp. *bulgaricus* B1 (0,39 cfu/cm^3.h), as compared with that in co-cultivation with *Lactobacillus delbrueckii* ssp. *bulgaricus* TAB2 (0,13 cfu/cm^3.h). The pathogen was more sensitive to the conditions created by

Lactobacillus delbrueckii ssp. *bulgaricus* B1. The values of the rate constant of dying were comparable (0,147 s^{-1} in co-cultivation with *Lactobacillus delbrueckii* ssp. *bulgaricus* B1 and 0,139 s^{-1} in co-cultivation with *Lactobacillus delbrueckii* ssp. *bulgaricus* TAB2). The high values of the reciprocal of the rate constant of dying of *Candida albicans* NBIMCC 74 (6.80 and 7.19, respectively) indicate that it has a considerable resistance to the conditions created by the two lactobacilli strains.

Table 1.

Kinetic parameters of the processes of single-strain culture and in a mixed population of the two *Lactobacillus delbrueckii* ssp. *bulgaricus* strains and *Candida albicans* NBIMCC 74

Strain	μ, h^{-1}	β, $cm^3/(cfu.h)$	k, s^{-1}	1/k, s
L. delbrueckii ssp.*bulgaricus* B1 blank	0,224	0,060		-
L. delbrueckii ssp. *bulgaricus* TAB2 blank	0,187	0,050	-	-
C. albicans NBIMCC 74 blank	0,223	0,017	-	
L. d. ssp. *bulgaricus* B1 (*L. d.* ssp. *bulgaricus* B1+ *C. albicans* NBIMCC 74)	0,125	0,011	-	-
C. albicans NBIMCC 74 (*L. d.* ssp. *bulgaricus* B1+ *C. albicans* NBIMCC 74)	0,060	0,390	0,147	6,800
L. d. ssp. *bulgaricus* TAB2 (*L. d.* ssp. *bulgaricus* TAB2+ *C. albicans* NBIMCC 74)	0,157	0,060	-	-
C. albicans NBIMCC 74 (*L. d.* ssp. *bulgaricus* TAB2+ *C. albicans* NBIMCC 74)	0,105	0,130	0,139	7,190

CONCLUSION

As a result of the experimental studies the following important conclusions can be resumed:

1. *Lactobacillus delbrueckii* ssp. *bulgaricus* B1 and *Lactobacillus delbrueckii* ssp. *bulgaricus* TAB2 maintain high concentration of viable cells in single-strain culture and in co-culture at a temperature of 37±1°C

2. Both *Lactobacillus delbrueckii* ssp. *bulgaricus* strains inhibited significantly the growth and development of *Candida albicans* NBIMCC 74. The observed antimicrobial activity was due to a great extent to the acidification of the medium because of the production and accumulation of lactic acide.

3. The demonstrated antimicrobial activity is a prerequisite for further research on the probiotic potential of the two *Lactobacillus delbrueckii* ssp. *bulgaricus* strains for their inclusion in the composition of probiotic preparations.

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This paper has been reviewed