

## ANTIMICROBIAL ACTIVITY OF *LACTOBACILLUS PLANTARUM* X2 AGAINST PATHOGENIC MICROORGANISMS

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### Abstract

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One of the requirements for a strain to be included in the composition of probiotic preparations is to exhibit antimicrobial activity against pathogenic microorganisms. The antimicrobial action of the strain *Lactobacillus plantarum* X2, isolated from naturally fermented sourdough, against pathogens is examined through co-cultivation of the *Lactobacillus* strain and each of the pathogens included in the study. The pathogenic microorganisms included in the present research are *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 8739, *Salmonella abony* NTCC 6017, *Salmonella* sp., *Staphylococcus aureus* ATCC 25293. It is observed that during separate cultivation of *Lactobacillus plantarum* X2 and each of the pathogens both *Lactobacillus plantarum* X2 and the pathogen accumulate high concentrations of viable cells. During co-cultivation *Lactobacillus plantarum* X2 maintains a high concentration of viable cells, while the number of living cells of the pathogen is reduced. The degree of reduction of the cells of the pathogen is strain specific and is partially due to the changes in the acidity of the medium as a result of the production of acids by *Lactobacillus plantarum* X2.

*Key words:* antimicrobial, co-cultivation, pathogen, *Escherichia coli*, *Salmonella*, *Staphylococcus*

### Introduction

Probiotics are live microorganisms that confer a beneficial effect on the host when administered in proper amounts (Kalliomaki et al., 2001; Brown and Valiere, 2004). As components of probiotics and probiotic foods can be used only strains of lactobacilli and bifidobacteria that are of human origin, resistant to gastric acid, bile and to the antibiotics, administered in medical practice, non-pathogenic; that have the potential to adhere to the gut epithelial tissue and produce antimicrobial substances; that allow the conduction of technological processes, in which high concentrations of viable cells are obtained, as well as industrial cultivation, encapsulation and freeze-drying and that remain active during storage (Mitsuoka, 1999; Kirtzalidou et al., 2011). A probiotic

strain should exhibit antimicrobial activity against pathogens, which is associated with inactivation of their enzyme systems, overcoming their adhesion, growth suppression and interference for their biological niche, as a result of which gastrointestinal microflora is normalized. This demands the selection of strains of lactobacilli and bifidobacteria with probiotic properties.

The purpose of this paper is to examine the antimicrobial activity of *Lactobacillus plantarum* X2 against pathogenic microorganisms.

### Materials and Methods

**Microorganisms.** In the present study are used: *Lactobacillus plantarum* X2, which is isolated from naturally fermented

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sourdough; test pathogenic microorganisms: *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 8739, *Salmonella* sp. (clinical isolation), *Salmonella abony* NTCC 6017 and *Staphylococcus aureus* ATCC 25293.

### Media

*Saline solution.* Composition (g.dm<sup>-3</sup>): NaCl - 5.

*LAPTg10-broth medium.* (Portillo et al., 1988).

*LAPTg10-agar medium.* Composition (g.dm<sup>-3</sup>): LAPTg10-broth medium; agar - 20. Coloured LAPTg10-agar medium is used for the present experiment. It is obtained by the addition of colouring agent after melting the medium prior to pouring it into plates.

*LBG-agar medium.* Composition (g.dm<sup>-3</sup>): tryptone - 10, yeast extract - 5, NaCl - 10, glucose - 10, agar - 20. pH is adjusted to 7.5.

### Determination of the antimicrobial activity against pathogens.

In the mixtures 0.5 ml of the 48-hour cultural suspension of the *Lactobacillus* strain, 0.5 ml of the suspension of the pathogen and 9 ml of the medium (LAPTg10-broth medium) are mixed, while in the control of the *Lactobacillus* strain and in the control of the pathogen 9.5 ml of the LAPTg10-broth medium are mixed with 0.5 ml of the suspension of the *Lactobacillus* strain or of the suspension of the pathogen, respectively. Joint cultivation of *Lactobacillus plantarum* X2 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 change of the titratable acidity and the concentration of viable cells of both the pathogen and the *Lactobacillus* strain, is performed. The number of viable cells is determined through appropriate tenfold dilutions of the samples and plating on LBG-agar medium (to determine the number of viable cells of the pathogen) and on coloured LAPTg10 - agar medium (to determine the number of viable cells of *Lactobacillus plantarum* X2). The Petri dishes are cultivated for 72 hours at 37±1°C until single colonies can be counted. The titratable acidity is determined using 0.1N NaOH. 5 cm<sup>3</sup> of each sample are mixed with 10 cm<sup>3</sup> dH<sub>2</sub>O and titrated with 0.1N NaOH, using phenolphthalein as an indicator, until the appearance of pale pink colour, which retains for 1 minute. The value for the titratable acidity is obtained by multiplying the millilitres 0.1N NaOH by the factor of the 0.1N NaOH and the number 20. Bacterial counts are transformed to log values.

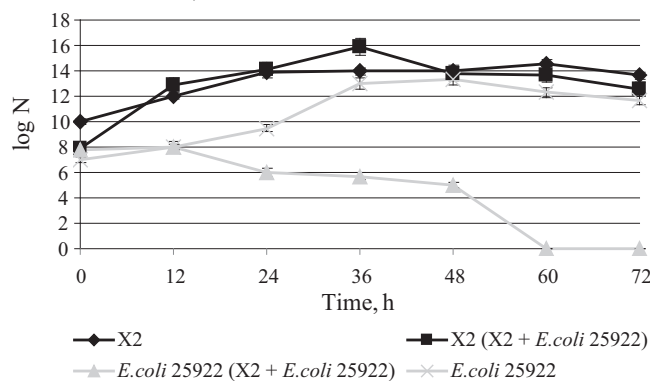
Results are shown as the average values and standard deviations obtained from three independent experiments. The Microsoft Excel 2007 is used for data analysis.

## Results and Discussion

In determining the antimicrobial activity of *Lactobacillus plantarum* X2 against pathogens in separate cultivation of

*Lactobacillus plantarum* X2 and each of the pathogenic microorganisms accumulation of high concentrations of viable cells of *Lactobacillus plantarum* X2 and of each of the pathogens is observed. But there are some differences when *Lactobacillus plantarum* X2 is co-cultivated with each of the pathogens.

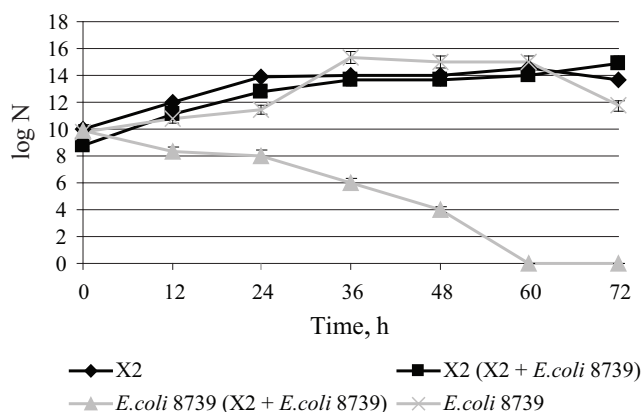
In co-cultivation of *Lactobacillus plantarum* X2 and *Escherichia coli* ATCC 25922, an increase in the concentration of viable cells of *Lactobacillus plantarum* X2 is observed, while that of the pathogen *Escherichia coli* ATCC 25922 is maintained during the first 12 hours, then the number of living cells of the pathogen is reduced and at the 60<sup>th</sup> hour no viable cells of the pathogen are found (Figure 1). The data is sustainable with the results, obtained with *Lactobacillus plantarum* NBIMCC 2415 and *Escherichia coli* ATCC 25922 at 37±1°C (Denkova and Nedelcheva, 2009).



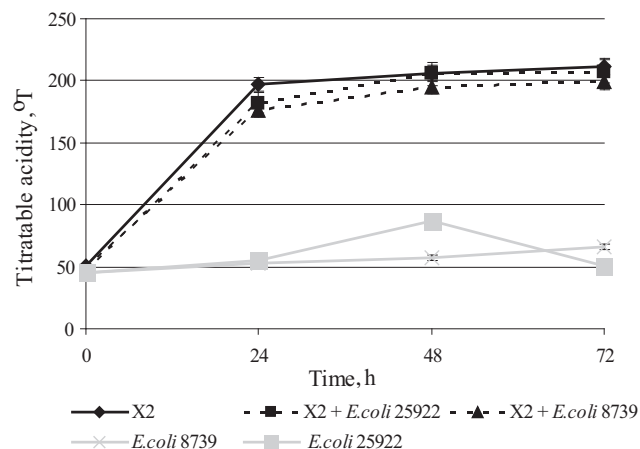
**Fig. 1. Survival of the cells of *Lactobacillus plantarum* X2 and *Escherichia coli* ATCC 25922 in separate cultivation and in a mixed population at 37 ± 1°C**

*Lactobacillus plantarum* X2 suppresses *Escherichia coli* ATCC 8739 from the beginning of the co-cultivation (Figure 2), while *Lactobacillus plantarum* NBIMCC 2415 inhibits the growth of the pathogen after the 12<sup>th</sup> hour of co-cultivation. Moreover, in the co-cultivation of *Escherichia coli* ATCC 25922 and *Lactobacillus plantarum* X2 no living cells of the pathogen are found at the 60<sup>th</sup> hour, while in the co-cultivation of *Escherichia coli* ATCC 8739 and *Lactobacillus plantarum* NBIMCC 2415 at the 72<sup>nd</sup> hour the number of living cells of the pathogen is about 10<sup>11</sup>cfu.cm<sup>-3</sup> (Denkova and Nedelcheva, 2009).

*Lactobacillus plantarum* NBIMCC 2415 inhibits *Salmonella abony* NTCC 6017 during co-cultivation but the decrease in the pathogen cell number starts after the 12<sup>th</sup> hour, while in co-cultivation of *Salmonella abony* NTCC 6017 and *Lactobacillus plantarum* X2 (Figure 4) the reduction of viable pathogen cells starts 12 hours later. However, the concentration of the pathogen at the 60<sup>th</sup> hour during the co-cultivation with *Lactobacillus plantarum* NBIMCC 2415 is about 10<sup>8</sup>cfu.cm<sup>-3</sup> (Denkova and Nedelcheva, 2009), while with *Lactobacillus plantarum* X2 it's 10<sup>4</sup>cfu.cm<sup>-3</sup>.



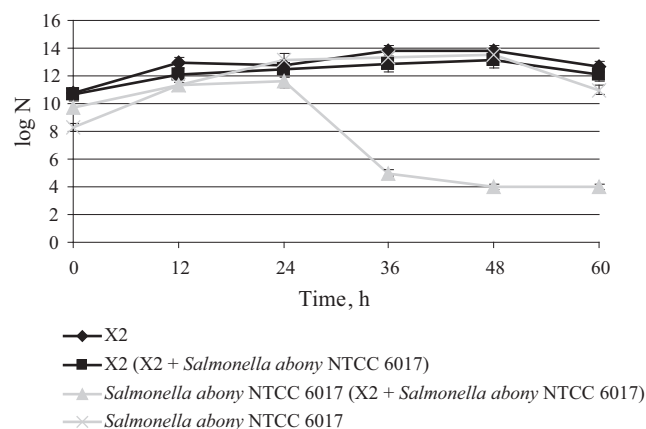
**Fig. 2.** Survival of cells of *Lactobacillus plantarum* X2 and *Escherichia coli* ATCC 8739 in separate cultivation and in a mixed population at  $37 \pm 1^\circ\text{C}$



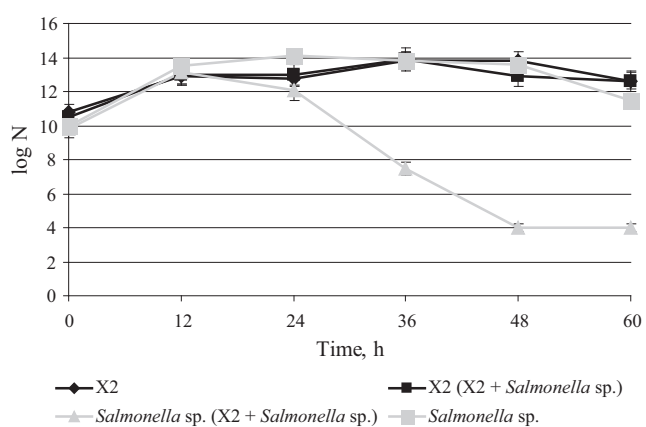
**Fig. 3.** Change in the titratable acidity in separate cultivation and in co-cultivation of *Lactobacillus plantarum* X2 with *Escherichia coli* 8739 or *Escherichia coli* ATCC 25922 at  $37 \pm 1^\circ\text{C}$

In co-cultivation of *Lactobacillus plantarum* X2 and *Salmonella* sp. (clinical isolate) the concentrations of viable cells of *Lactobacillus plantarum* X2 and of *Salmonella* sp. increase during the first 12 hours, after which the concentration of viable cells of *Lactobacillus plantarum* X2 continues increasing while that of the pathogen is reduced rapidly, reaching  $10^4 \text{cfu} \cdot \text{cm}^{-3}$  at the 60<sup>th</sup> hour (Figure 5). The same effect is observed in co-cultivation of *Lactobacillus plantarum* NBIMCC 2415 and the same clinical isolate of *Salmonella* sp. – the decrease of the pathogen cell number starts after the 12<sup>th</sup> hour, but at the 60<sup>th</sup> hour of co-cultivation there are no living pathogen cells (Denkova and Nedelcheva, 2009).

In co-cultivation the concentrations of viable cells of *Lactobacillus plantarum* X2 and of *Staphylococcus aureus*



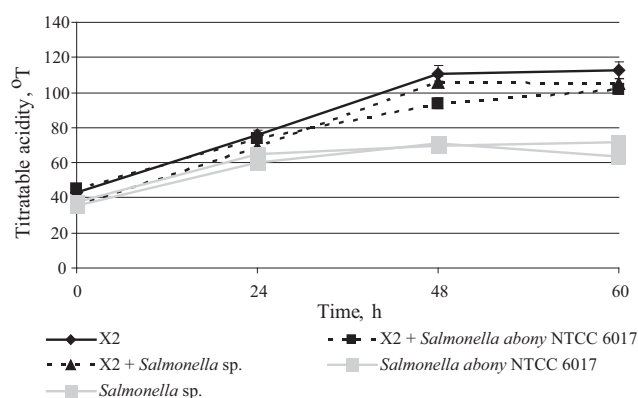
**Fig. 4.** Survival of the cells of *Lactobacillus plantarum* X2 and *Salmonella abony* NTCC 6017 in separate cultivation and in a mixed population at  $37 \pm 1^\circ\text{C}$



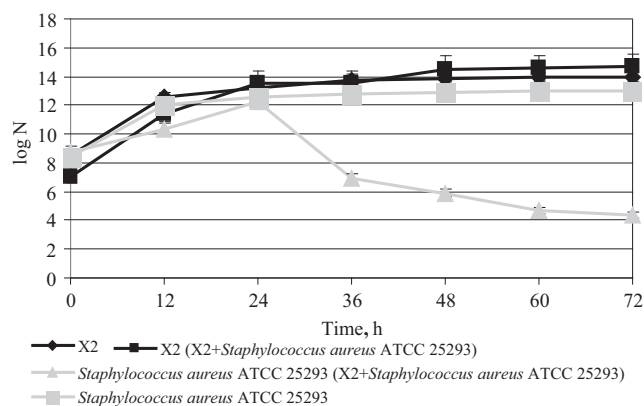
**Fig. 5.** Survival of the cells of *Lactobacillus plantarum* X2 and *Salmonella* sp. in separate cultivation and cultivation in a mixed population at  $37 \pm 1^\circ\text{C}$

ATCC 25293 increase during the first 24 hours. After that the concentration of viable cells of *Lactobacillus plantarum* X2 continues growing, while that of the pathogen is reduced, reaching  $2.4 \times 10^4 \text{cfu} \cdot \text{cm}^{-3}$  at the 72<sup>nd</sup> hour (Figure 7). In contrast, during co-cultivation of *Lactobacillus plantarum* NBIMCC 2415 and *Staphylococcus aureus* ATCC 25293 at  $37 \pm 1^\circ\text{C}$ , the reduction of living cells of the pathogen starts after the 6<sup>th</sup> hour (Denkova and Nedelcheva, 2009), but the pathogen cell numbers at the 60<sup>th</sup> hour of co-cultivation with *Lactobacillus plantarum* NBIMCC 2415 and *Lactobacillus plantarum* X2 are the same –  $2.4 - 4 \times 10^4 \text{cfu} \cdot \text{cm}^{-3}$ .

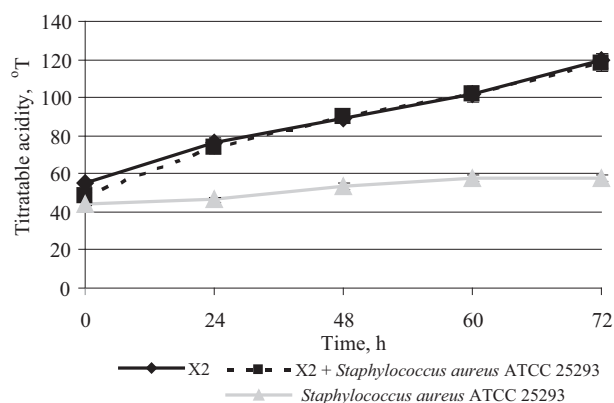
The titratable acidity of the controls of the pathogens is significantly lower compared to the control of the *Lactobacillus* strain and the five mixtures (*Lactobacillus plantarum* X2 and *Escherichia coli* ATCC 25922; *Lactobacillus plantarum* X2 and *Escherichia coli* 8739; *Lactobacillus planta-*



**Fig. 6.** Change in the titratable acidity of the medium in separate cultivation and in cultivation in a mixed population of *Lactobacillus plantarum* X2 and *Salmonella abony* NTCC 6017 or *Salmonella sp.* at  $37 \pm 1^\circ\text{C}$



**Fig. 7.** Change in the titratable acidity of the medium in separate cultivation and in cultivation in a mixed population of *Lactobacillus plantarum* X2 with *Staphylococcus aureus* ATCC 25293 at  $37 \pm 1^\circ\text{C}$



**Fig. 8.** Survival of the cells of *Lactobacillus plantarum* X2 and *Staphylococcus aureus* ATCC 25293 in separate cultivation and in cultivation in a mixed population at  $37 \pm 1^\circ\text{C}$

*rum* X2 and *Salmonella abony* NTCC 6017; *Lactobacillus plantarum* X2 and *Salmonella sp.*; *Lactobacillus plantarum* X2 and *Staphylococcus aureus* ATCC 25293) (Figures 3, 6 and 8). These results are sustainable with the ones, obtained with *Lactobacillus plantarum* NBIMCC 2415 and the same pathogens at  $37 \pm 1^\circ\text{C}$  (Denkova and Nedelcheva, 2009).

*Lactobacillus plantarum* X2 demonstrates greater inhibitory activity against *Escherichia coli* ATCC 8739, *Salmonella abony* NTCC 6017 and *Staphylococcus aureus* ATCC 25293, but lower against *Salmonella sp.* than *Lactobacillus plantarum* NBIMCC 2415 (Denkova and Nedelcheva, 2009).

## Conclusion

*Lactobacillus plantarum* X2 demonstrates antimicrobial activity against *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 8739, *Salmonella abony* NTCC 6017, *Salmonella sp.*, *Staphylococcus aureus* ATCC 25293. During co-cultivation *Lactobacillus plantarum* X2 retains a high concentration of viable cells, while the cell count of the pathogen is reduced and the degree of reduction is strain specific and is partially due to the changes in the acidity of the medium as a result of the production of acids by *Lactobacillus plantarum* X2. The antimicrobial activity against pathogens of *Lactobacillus plantarum* X2 allows its application as a biopreservative in foods and makes it a potentially probiotic strain, which after further research can be incorporated in the composition of probiotics for prevention and treatment.

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