



ANTIMICROBIAL ACTIVITY OF THE PROBIOTIC STRAIN *LACTOBACILLUS DELBRUECKII* SSP. *BULGARICUS* GB OF HUMAN ORIGIN AGAINST PATHOGENS

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Received May 9th 2013, accepted 15th June 2013

Abstract: Probiotics help restore the balance of the gastrointestinal microflora, but only strains with certain properties can be included in their formulations. One of the requirements for a strain to be probiotic is to exhibit inhibitory activity against pathogens. The antimicrobial activity of the strain *Lactobacillus delbrueckii* ssp. *bulgaricus* GB against the pathogens *E.coli* ATCC 25922, *E.coli* ATCC 8739, *Salmonella abony* NTCC 6017, *Salmonella* sp., *Staphylococcus aureus* ATCC 25293 and *Proteus vulgaris* J is determined by joint cultivation at $37\pm 1^\circ\text{C}$. *Lactobacillus delbrueckii* ssp. *bulgaricus* GB inhibits the growth of the pathogens - for 60 - 72 hours the numbers of viable cells of the pathogens are reduced. It has been shown that the changes in the proportions in the mixed populations are due to the accumulation of lactic and other organic acids which acidify the medium and change the conditions for the growth of the pathogens, leading to reduction of the number of viable cells of the pathogens. The demonstrated inhibitory activity of *Lactobacillus delbrueckii* ssp. *bulgaricus* GB makes the strain potentially probiotic and after additional studies it can be included in the composition of probiotics and functional foods.

Keywords: Joint cultivation, Probiotic, *E.coli*, *Salmonella*, *Staphylococcus*, *Proteus*

1. Introduction

Maintaining the balance of the gastrointestinal microflora is necessary for good health. In order to restore the balance of the intestinal microflora it is necessary to consummate food and concentrates containing beneficial lactobacilli and bifidobacteria, known as functional foods and probiotics, respectively. According to FAO probiotics are live microorganisms that have beneficial effect on the host when administered in adequate amounts [1, 2].

The main components of probiotics are lactic acid bacteria (*Lactobacillus*, *Enterococcus*, *Pediococcus*, *Lactococcus*, *Streptococcus*, *Leuconostoc*) and bifidobacteria. They are also applied in the manufacture of probiotic foods [3, 4, 5], the largest proportion being the lactobacilli. But only lactobacilli with certain properties can be included in the composition of probiotics and probiotic foods [4, 5]: to be a part of the natural microflora in humans and animals; to be able to suppress and expel pathogenic and toxicogenic microorganisms from the

biological niche; to allow industrial cultivation; to have antimicrobial activity against conditionally pathogenic, carcinogenic and pathogenic microorganisms; to have the ability to adhere to epithelial cells or cell lines; to be able to survive under the conditions in the stomach and the intestines (acidic pH in the stomach and bile) [8, 9]; to be able to reproduce in the intestinal tract; to produce antimicrobial substances; to modulate the immune response and to be safe for clinical and food applications.

The studies of Saxelin et al., (1996 a, b), Donohue & Salminen, (1996), Salminen et al., (1998) [3, 4, 5, 10, 11, 12] demonstrate the safety of lactic acid bacteria and bifidobacteria and strains belonging to the genera *Lactobacillus*, *Lactococcus* and *Bifidobacterium* most often are assigned with GRAS status.

The purpose of the present paper is to determine the antimicrobial activity of a probiotic strain of human origin *Lactobacillus delbrueckii* ssp. *bulgaricus* GB against the following pathogens: *E.coli* ATCC 25922, *E.coli* ATCC 8739, *Salmonella abony* NTCC 6017, *Salmonella* sp., *Staphylococcus aureus* ATCC 25293 and *Proteus vulgaris* J.

2. Materials and Methods

1. Media

Sterile skimmed milk with titrable acidity 16-18°T. Composition (g/dm³): skimmed milk powder (Scharlau). Sterilization - 15 minutes at 118°C.

Saline solution. Composition (g/dm³): NaCl - 5. Sterilization - 20 minutes at 121°C.

LAPTg10-broth. Composition (g/dm³): peptone - 15, yeast extract - 10; tryptone - 10, glucose - 10. pH is adjusted to 6.6 - 6.8 and Tween 80 - 1cm³/dm³ is added. Sterilization - 20 minutes at 121°C.

LAPTg10-agar. Composition (g/dm³): LAPTg10-broth + 2% agar. Sterilization - 20 minutes at 121°C.

LBG-agar. Composition (g/dm³): Tryptone - 10, yeast extract - 5, NaCl - 10, glucose - 10, agar - 20. pH is adjusted to 7.5. Sterilization - 20 minutes at 121°C.

2. Determination of the antimicrobial activity against pathogenic microorganisms

To determine the antimicrobial activity of *Lactobacillus delbrueckii* ssp. *bulgaricus* GB against pathogens a 48 hour culture of the *Lactobacillus* strain is used. In the mixtures are mixed 0.5 cm³ of the suspension of *Lactobacillus delbrueckii* ssp. *bulgaricus* GB, 0.5 cm³ of the suspension of the pathogen and 9 cm³ of culture medium (skimmed milk), and in the controls of the *Lactobacillus* strain or the pathogen 9.5 cm³ culture medium (skimmed milk) is mixed with 0.5 cm³ of the suspension of *Lactobacillus delbrueckii* ssp. *bulgaricus* GB or of the pathogen, respectively. Joint cultivation of *Lactobacillus delbrueckii* ssp. *bulgaricus* GB and each of pathogens *E.coli* ATCC 25922, *E.coli* ATCC 8739, *Salmonella abony* NTCC 6017, *Salmonella* sp., *Staphylococcus aureus* ATCC 25293 and *Proteus vulgaris* J under static conditions in a thermostat at 37±1°C for 72 hours, taking samples at the 0th, 12th, 24th, 36th, 48th, 60th and 72nd hour is conducted and the change in the titrable acidity and the concentration of viable cells of the pathogen and of *Lactobacillus delbrueckii* ssp. *bulgaricus* GB is monitored.

3. Processing of the results

Data from triplicate experiments is processed using the software MS Office Excel 2003 and Origin Pro 8.1, using statistical functions to determine the standard deviation and maximum error of assessment in the significance level of $\alpha < 0,05$.

3. Results and Discussion

The antimicrobial activity of *Lactobacillus delbrueckii* ssp. *bulgaricus* GB against the pathogens *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 8739, *Salmonella* sp. (clinical isolate), *Salmonella abony* NTCC 6017, *Staphylococcus aureus* ATCC 25293, *Proteus vulgaris* J during joint and separate cultivation at $37\pm 1^\circ\text{C}$ in skimmed milk is determined.

During separate cultivation *Lactobacillus delbrueckii* ssp. *bulgaricus* GB and the pathogens *E.coli* ATCC 25922 and *E.coli* ATCC 8739 for 12 to 24 hours at $37\pm 1^\circ\text{C}$ accumulate high concentrations of viable cells. These high concentrations of living cells preserve *Lactobacillus delbrueckii* ssp. *bulgaricus* GB in a mixed population with the pathogen. In joint cultivation of the *Lactobacillus* strain and *E.coli* ATCC 25922 at static conditions, an increase in the concentration of viable cells of *Lactobacillus delbrueckii* ssp. *bulgaricus* GB is detected, while that of *E.coli* ATCC 25922 starts to decrease after the first 12 hours and at the 60th hour no viable cells of the pathogen are established (Fig. 1).

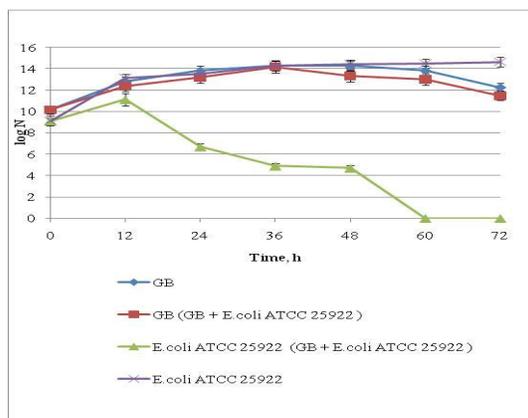


Fig. 1. Survival of *Lactobacillus delbrueckii* ssp. *bulgaricus* GB and *E.coli* ATCC 25922 during separate cultivation and cultivation in a mixed population at $37\pm 1^\circ\text{C}$.

In the study of the inhibitory activity of *Lactobacillus delbrueckii* ssp. *bulgaricus* GB against *E.coli* ATCC 8739 the concentrations of viable cells of *Lactobacillus delbrueckii* ssp. *bulgaricus* GB and of the pathogen *E.coli* ATCC 8739 increase in the first 12 hours, which is consistent with the results obtained with *E.coli* ATCC 25922. After that the concentration of viable lactobacilli cells retains while that the pathogen is quickly reduced and at the 60th hour there are no living cells of the pathogen, similarly to the joint cultivation with *E.coli* ATCC 25922 (Fig. 1 and Fig. 2).

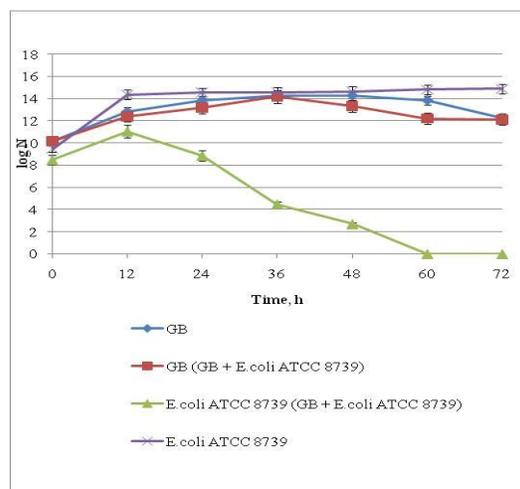


Fig. 2. Survival of *Lactobacillus delbrueckii* ssp. *bulgaricus* GB and *E.coli* ATCC 8739 during separate cultivation and cultivation in a mixed population at $37\pm 1^\circ\text{C}$.

In tracking the change in the titratable acidity it is noticeable that the acidity values of both the two controls of the pathogens are significantly lower in comparison to the control of the *Lactobacillus* strain and of that of the two mixtures (*Lactobacillus delbrueckii* ssp. *bulgaricus* GB and *E.coli* ATCC 25922; *Lactobacillus delbrueckii* ssp. *bulgaricus* GB and *E.coli* ATCC 8739) and the titratable acidity values of the

mixtures are lower than the values of the control of *Lactobacillus delbrueckii* ssp. *bulgaricus* GB for each hour of sampling, which shows that the inhibition of the pathogen by *Lactobacillus delbrueckii* ssp. *bulgaricus* GB is due to a great extent to the acidification of the medium (Fig. 3).

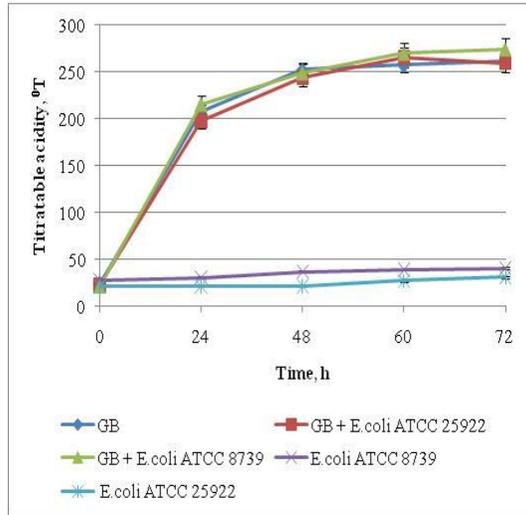


Fig. 3. Change of the titratable acidity of the medium during separate cultivation and cultivation in a mixed population of *Lactobacillus delbrueckii* ssp. *bulgaricus* GB and *E. coli* ATCC 8739 or *E. coli* ATCC 25922 at $37\pm 1^\circ\text{C}$.

During the cultivation of *Lactobacillus delbrueckii* ssp. *bulgaricus* GB and *Salmonella* sp. (clinical isolate) at $37\pm 1^\circ\text{C}$, an increase in the concentration of viable cells of *L.d.ssp.bulgaricus* GB as well as of *Salmonella* sp. during the first 12 hours is observed, after which the concentration of viable lactobacilli cells continues to increase, while that of the pathogen is reduced and by the 72nd hour no living cells of *Salmonella* sp. are defined (Fig. 4).

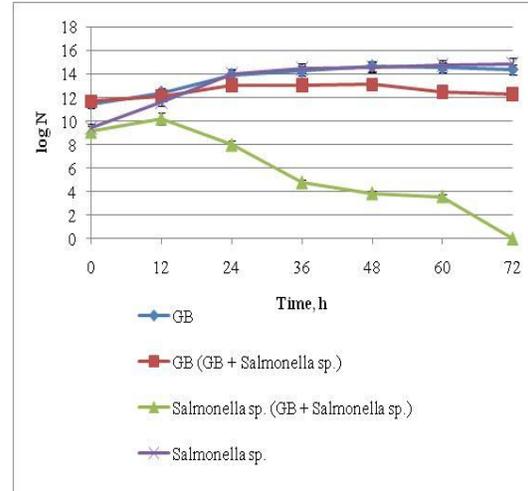


Fig. 4. Survival of *Lactobacillus delbrueckii* ssp. *bulgaricus* GB and *Salmonella* sp. during separate cultivation and cultivation in a mixed population at $37\pm 1^\circ\text{C}$.

In the joint development of *Salmonella abony* NTCC 6017 and *Lactobacillus delbrueckii* ssp. *bulgaricus* GB an increase of the cell concentration of the *Lactobacillus* strain and of the pathogen in the first 12 hours is observed.

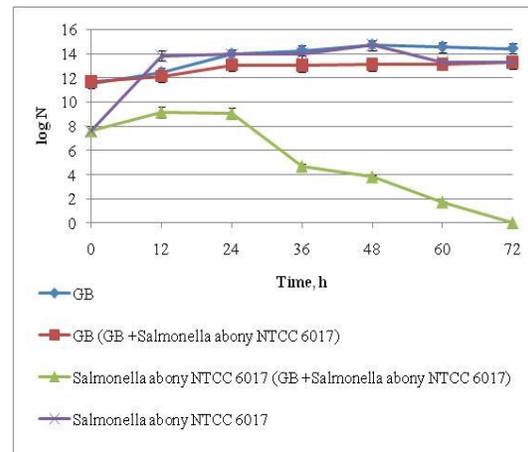


Fig. 5. Survival of *Lactobacillus delbrueckii* ssp. *bulgaricus* GB and *Salmonella abony* NTCC 6017 during separate cultivation and cultivation in a mixed population at $37\pm 1^\circ\text{C}$.

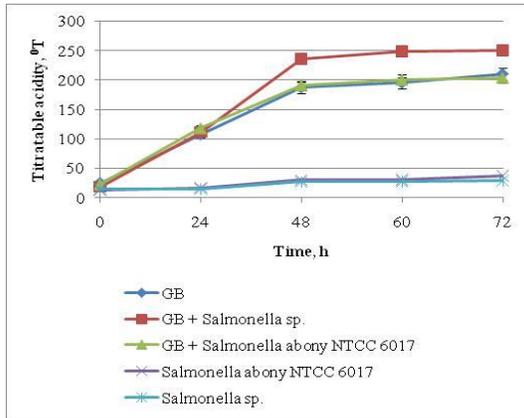


Fig. 6. Change in the titratable acidity of the medium during separate cultivation and cultivation in a mixed population of *Lactobacillus delbrueckii* ssp. *bulgaricus* GB and *Salmonella abony* NTCC 6017 or *Salmonella* sp. at $37\pm 1^\circ\text{C}$.

From the 12th to the 60th hour the concentration of lactic acid bacteria continues to increase while that of *Salmonella abony* NTCC 6017 decreases reaching 0 at the 72nd hour as in the study of the antimicrobial activity of *Lactobacillus delbrueckii* ssp. *bulgaricus* GB against *Salmonella* sp. (Fig. 4 and Fig. 5), which is mainly a result of the acidification of the medium, due to the accumulation of lactic acid and other organic acids (Fig. 6).

In the separate cultivation of *Lactobacillus delbrueckii* ssp. *bulgaricus* GB and *Staphylococcus aureus* ATCC 25293 both strains accumulate high concentration of active cells. During the joint cultivation *Lactobacillus delbrueckii* ssp. *bulgaricus* GB and *Staphylococcus aureus* ATCC 25293 a decrease in the concentration of the pathogen, starting after 12th hour is observed. As in the determination of the antimicrobial activity against the two strains of *Escherichia coli*, *Salmonella* sp. and *Salmonella abony* NTCC 6017, the number of living cells of this pathogen is reduced completely under the action of

Lactobacillus delbrueckii ssp. *bulgaricus* GB. At the end of the process no living cells of the pathogen are detected (Fig. 7), mainly due to the lowering of the pH as a result of the increase in the titratable acidity (Fig. 8).

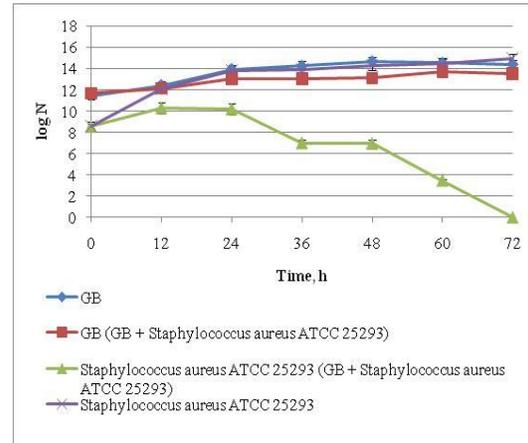


Fig. 7. Survival of *Lactobacillus delbrueckii* ssp. *bulgaricus* GB and *Staphylococcus aureus* ATCC 25293 during separate cultivation and cultivation in a mixed population at $37\pm 1^\circ\text{C}$.

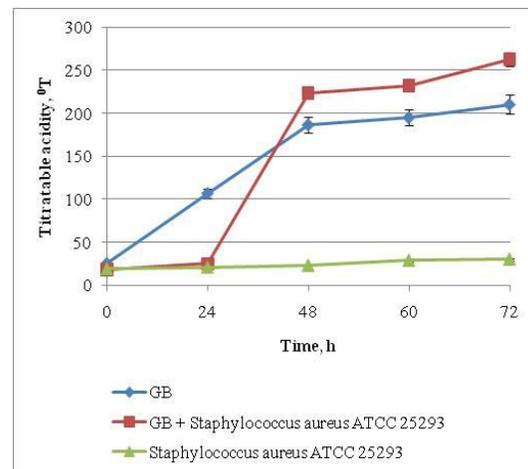


Fig. 8. Change in the titratable acidity of the medium during separate cultivation and cultivation in a mixed population of *Lactobacillus delbrueckii* ssp. *bulgaricus* GB and *Staphylococcus aureus* ATCC 25293 at $37\pm 1^\circ\text{C}$.

In studying the antimicrobial activity of *Lactobacillus delbrueckii* ssp. *bulgaricus* GB against *Proteus vulgaris* J the concentration of viable cells of *Lactobacillus delbrueckii* ssp. *bulgaricus* GB and of the pathogen increases in the first 12 hours. After that the number of living cells of the *Lactobacillus* strain continues to grow at a slower rate, while the cell number of the pathogen is reduced, reaching 0 at the 60th hour (Fig. 9).

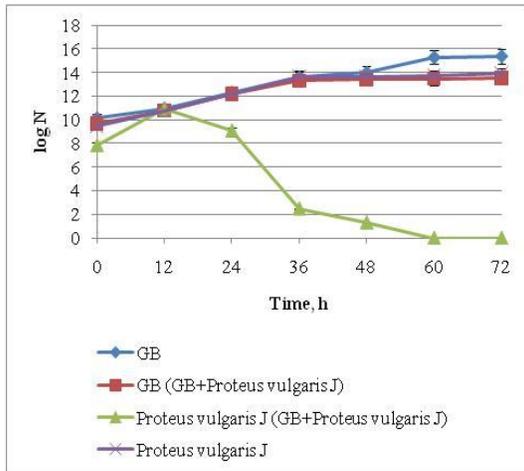


Fig. 9. Survival of *Lactobacillus delbrueckii* ssp. *bulgaricus* GB and *Proteus vulgaris* J during separate cultivation and cultivation in a mixed population at 37±1°C.

For each sampling the acidity of the control of the pathogen is lower than that of the control of the *Lactobacillus* strain and that of the mixture, which again indicates that the inhibition of pathogens by lactobacilli is largely a result of the decrease of the pH of the medium, resulting from the accumulation of organic acids produced by the lactobacilli (Fig. 10).

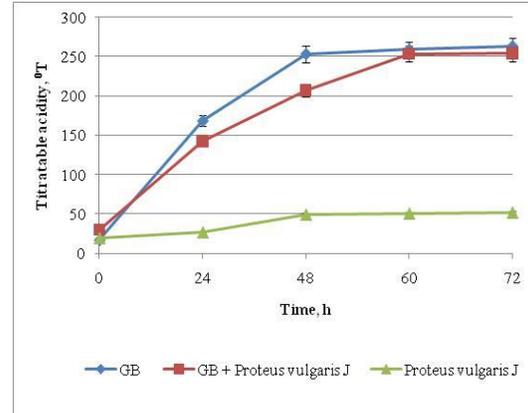


Fig. 10. Change in the titratable acidity of the medium during separate cultivation and cultivation in a mixed population of *Lactobacillus delbrueckii* ssp. *bulgaricus* GB and *Proteus vulgaris* J at 37±1°C.

4. Conclusion

The strain *Lactobacillus delbrueckii* ssp. *bulgaricus* GB inhibits the growth of the pathogens *E.coli* ATCC 25922, *E.coli* ATCC 8739, *Salmonella abony* NTCC 6017, *Salmonella* sp., *Staphylococcus aureus* ATCC 25293 and *Proteus vulgaris* J. In joint cultivation of *Lactobacillus delbrueckii* ssp. *bulgaricus* GB and all of the pathogens the *Lactobacillus* strain retains a high concentration of viable cells, while the cell number of the pathogen is reduced, the degree of reduction being strainspecific and partly due to the change in the acidity of the medium as a result of the acid production by *Lactobacillus delbrueckii* ssp. *bulgaricus* GB. The antimicrobial activity against pathogens makes the tested strain a potentially probiotic one, which after further studies may be included in the composition of probiotic preparations for prophylaxis and treatment.

5. References

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