Antimicrobial activity of lactobacilli of human origin against Pseudomonas aeruginosa

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Antimicrobial activity of lactobacilli of human origin against Pseudomonas aeruginosa: The antimicrobial activity of 3 strains from the genus Lactobacillus – L.acidophilus A2, L.acidophilus Ac and L.delbrueckii ssp. bulgaricus GB – of human origin against Pseudomonas aeruginosa NBIMCC 1390 is determined by joint cultivation at 37±1°C. It is established that the Lactobacillus strains inhibit the growth of Pseudomonas aeruginosa NBIMCC 1390 and at the 60th hour of joint cultivation there are no viable pathogen cells. It has been demonstrated that the changes in the proportions of the microorganisms in the mixed population (each lactobacilli strain and the pathogen) result from the production and accumulation of lactic and other organic acids that acidify the medium and modify the growth conditions for the pathogen, leading to the total reduction of living cells of Pseudomonas aeruginosa NBIMCC 1390.

Key words: Lactobacillus, Pseudomonas, joint cultivation, antimicrobial activity

INTRODUCTION

Probiotics are living microorganisms that confer beneficial effects to the host when administered in adequate amounts [1, 4]. They have proven beneficial effects in gastrointestinal infections, reduction of serum cholesterol, protecting the immune system, inhibition of infections caused by Helicobacter pylori, Crohn's disease, restoration of the microflora in the stomach and intestine after antibiotic treatment. They also demonstrate anti-cancer properties, antimutagenic effect, antidiarrheal properties and others [2, 8].

Lactobacilli and bifidobacteria are natural components of the gastrointestinal microflora of a healthy person and they maintain its balance. They are included in the composition of probiotics and probiotic foods [3, 6, 7].

Not all Lactobacillus and Bifidobacterium strains can be used as components of probiotics or probiotic foods, but only those which meet the following requirements: 1) to be of human origin; 2) to be non-pathogenic; 3) to be resistant to gastric juice and bile salts; 4) to allow the implementation of technological processes aiming at accumulation of high concentrations of viable cells; 5) to have the potential to adhere to the gastrointestinal epithelium; 6) to produce antimicrobial substances; 7) to be resistant to the antibiotics applied in medical practice; 8) to allow industrial cultivation, encapsulation, freeze-drying and to retain activity during storage and 9) to be safe for clinical and food applications [5]. This requires the mandatory selection of bifidobacteria and lactobacilli strains with probiotic properties.

One of the requirements for probiotic strains is to possess antimicrobial activity against conditionally pathogenic, carcinogenic and pathogenic microorganisms, which is associated with inactivation of their enzyme systems, overcoming their adhesion, inhibiting their growth and expulsion of the biological niche resulting in the normalization of the balance of the gastrointestinal microflora.

The purpose of the present study is to determine the antimicrobial activity of Lactobacillus acidophilus A2, Lactobacillus acidophilus Ac and Lactobacillus delbrueckii ssp.bulgaricus GB of human origin against Pseudomonas aeruginosa by joint cultivation.

MATERIALS AND METHODS

Microorganisms:
Lactobacillus acidophilus A2, Lactobacillus acidophilus Ac and Lactobacillus delbrueckii ssp.bulgaricus GB of human origin;
Pseudomonas aeruginosa NBIMCC 1390.
Media:
Saline solution. Composition (g/dm$^3$): NaCl - 5. Sterilization - 20 minutes at 121ºC.
Sterile skimmed milk with titratable acidity 16-18°T
LAPTg10-agar. Composition (g/dm$^3$): peptone - 15, yeast extract - 10; tryptone - 10, glucose – 10, agar - 20. pH is adjusted to 6.6 - 6.8 and Tween 80 - 1 cm$^3$/dm$^3$ is added. Sterilization - 20 minutes at 121ºC.
LBG-agar. Composition (g/dm$^3$): Tryptone - 10 g, yeast extract - 5, NaCl – 10, glucose – 10, agar - 20. pH is adjusted to 7.5. Sterilization - 20 minutes at 121ºC.

Determination of inhibitory activity against pathogenic microorganisms
In the mixtures 0.5 cm$^3$ of the 48-hour cultural suspension of the Lactobacillus strain, 0.5 cm$^3$ of the suspension of the pathogen and 9 cm$^3$ of the medium (skimmed milk) are mixed, while in the control of the Lactobacillus strain and in the control of the pathogen 9.5 ml of skimmed milk are mixed with 0.5 cm$^3$ of the suspension of the Lactobacillus strain or the suspension of the pathogen, respectively. Joint and separate cultivation of each of the Lactobacillus strains and the pathogen Pseudomonas aeruginosa NBIMCC 1390 under static conditions in a thermostat at 37±1ºC for 72 hours is performed. Samples are taken at 0, 12, 24, 36, 48, 60 and 72 h and the change of the titratable acidity and the concentration of viable cells of both the pathogen and the Lactobacillus strain are monitored. The number of viable cells is determined through appropriate serial dilutions of the samples and spread plating on LBG-agar medium (to determine the number of viable cells of Pseudomonas aeruginosa NBIMCC 1390) and on coloured LAPTg10 – agar medium (to determine the number of viable cells of the Lactobacillus strains). The Petri dishes are incubated for 72 hours at 37±1ºC until the appearance of countable single colonies. The count of the colonies is then used to estimate the number of bacteria in the original sample. Bacterial counts are transformed to log values. The titratable acidity is determined using 0.1N NaOH. 5 cm$^3$ of each sample are mixed with 10 cm$^3$ dH$^2$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 milliliters 0.1N NaOH by the factor of the 0.1N NaOH and the number 20.

RESULTS AND DISCUSSION
In studying the inhibitory activity of Lactobacillus acidophilus A2 against Pseudomonas aeruginosa NBIMCC 1390 a reduction in the viable cell concentration of the pathogen from the beginning of the joint cultivation is observed. The number of living cells of the pathogen reaches 0 at the 60th hour while the concentration of the Lactobacillus acidophilus A2 increases to $10^{14}$ cfu/cm$^3$ (Fig. 1).

![Fig. 1. Survival of the cells of Lactobacillus acidophilus A2 and Pseudomonas aeruginosa NBIMCC 1390 in separate cultivation and in a mixed population at 37±1ºC](image-url)
In tracking the changes in the titratable acidity it is noticeable that the titratable acidity values increase significantly in the presence of lactobacilli. The high acidity of the medium of the mixture is a result of the synthesis of lactic and other organic acids by the *Lactobacillus* strain in the mixed population and is associated with the reduction of viable cells of *Pseudomonas aeruginosa* NBIMCC 1390 (Fig. 2).

![Fig. 2. Change in the titratable acidity in separate cultivation and in joint cultivation of *Lactobacillus acidophilus* A2 and *Pseudomonas aeruginosa* NBIMCC 1390 at 37±1°C](image)

In the joint cultivation of *Pseudomonas aeruginosa* NBIMCC 1390 and *Lactobacillus acidophilus* Ac there is an increase in the cellular concentration of both the *Lactobacillus* strain and the pathogen in the first 12 hours. From the 12th to the 72nd hour the concentration of lactic acid bacteria remains almost unchanged, while that of *Pseudomonas aeruginosa* NBIMCC 1390 decreases and at the 60th hour no living cells of the pathogen are defined (Fig. 3).

![Fig. 3. Survival of the cells of *Lactobacillus acidophilus* Ac and *Pseudomonas aeruginosa* NBIMCC 1390 in separate cultivation and in a mixed population at 37±1°C](image)

The complete reduction of viable cells of the pathogen during the joint cultivation is largely a result of the increase in the titratable acidity values, which in its turn is due to the accumulation of lactic acid and other organic acids in the medium (Fig. 4).

In the joint cultivation of *Lactobacillus delbrueckii* ssp. *bulgaricus* GB and *Pseudomonas aeruginosa* NBIMCC 1390 at 37±1°C an increase in the concentration of viable cells of *Lactobacillus delbrueckii* ssp. *bulgaricus* GB as well as of *Pseudomonas aeruginosa* NBIMCC 1390 in the first 12 hours is established. After that the concentration of viable lactobacilli cells keeps increasing, while that of the pathogen is reduced and by the 60th hour no living cells of *Pseudomonas aeruginosa* NBIMCC 1390 are defined (Fig.
5). The observed reduction in the cellular pathogen concentration to a great extent is due to the increased acidity of the medium in the mixtures (Fig. 6).

**Fig. 4.** Change in the titratable acidity in separate cultivation and in joint cultivation of *L. acidophilus* Ac and *Pseudomonas aeruginosa* NBIMCC 1390 at 37±1°C

**Fig. 5.** Survival of the cells of *L. d. ssp. bulgaricus* GB and *Pseudomonas aeruginosa* NBIMCC 1390 in separate cultivation and in a mixed population at 37±1°C

**Fig. 6.** Change in the titratable acidity in separate cultivation and in joint cultivation of *L. d. ssp. bulgaricus* GB and *Pseudomonas aeruginosa* NBIMCC 1390 at 37±1°C

**CONCLUSION**

*Lactobacillus acidophilus* A2, *Lactobacillus acidophilus* Ac and *Lactobacillus delbrueckii* ssp. *bulgaricus* GB exhibit significant antimicrobial activity against *Pseudomonas aeruginosa* NBIMCC 1390. During the joint cultivation of each of the strains *Lactobacillus acidophilus* A2, *Lactobacillus acidophilus* Ac or *Lactobacillus delbrueckii* ssp. *bulgaricus* GB and the pathogen the *Lactobacillus* strains maintain high concentrations of viable cells, while the number of cells of the pathogen is reduced. The degree of reduction
of the pathogen cell concentration is strain specific and is partly due to the changes in the acidity of the medium as a result of the production of acids by the lactobacilli strains. The exhibited antimicrobial activity against *Pseudomonas aeruginosa* NBIMCC 1390 makes *Lactobacillus acidophilus* A2, *Lactobacillus acidophilus* Ac and *Lactobacillus delbrueckii* ssp. *bulgaricus* GB potentially probiotic strains.

**REFERENCES**


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This paper has been reviewed