

Probiotic properties of *Lactobacillus plantarum* BG24, isolated from naturally fermented cereal beverage

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Probiotic properties of *Lactobacillus plantarum* BG24, isolated from naturally fermented cereal beverage. *Lactobacillus plantarum* BG24 was isolated from naturally fermented cereal beverage - boza and identified by molecular-genetic methods (sequencing). The stability of the cells of the strain in artificial gastric juice and bile - at pH=2 + pepsin, pH=4.5 + pancreatin and pH=7 + pancreatin and at different concentrations of bile salts (0,15%; 0,3%; 0,6% and 1%) in the medium was examined. It has been shown that the strain survived in the conditions of the gastrointestinal tract, maintaining high concentration of active cells. The antibiotic resistance of the strain to the most commonly used antibiotics in clinical practice was studied. It has been shown that the strain is resistant to the majority of the tested antibiotics.

Key words: *Lactobacillus plantarum*, pH, bile salts, boza, cereal, antibiotic resistance

INTRODUCTION

The health status of the individuals is determined by the microflora inhabiting the gastrointestinal tract. Diet, stress, diseases, treatments and the environment influence the composition and activity of microorganisms in the gut. One way to restore the balance of the gastrointestinal microflora is the administration of beneficial lactobacilli and bifidobacteria in the form of probiotics or probiotic foods.

Probiotics are live microorganisms that confer beneficial effects to the host, when administered in adequate amounts [2, 7]. Their beneficial effects in gastro-intestinal infections, reduction of serum cholesterol, protecting the immune system, suppression of infections caused by *Helicobacter pylori*, Krohn's disease, restoring the microflora in the gut and intestine after antibiotic treatment, their anti-cancer properties, antimutagenic effect, antiarrheal properties and others are well known [1, 8, 10, 12].

The main components of probiotics are lactic acid bacteria (*Lactobacillus*, *Enterococcus*, *Pediococcus*, *Lactococcus*, *Streptococcus*, *Leuconostoc*) and bifidobacteria that are applied in the manufacture of probiotic foods as well [7, 10, 11], the largest proportion being the lactobacilli.

Not all the lactobacilli can be included in the composition of the probiotics and probiotic foods, but only those that have certain characteristics [9, 10, 13]: to be part of the natural microflora in humans and animals; to adhere to epithelial cells or cell lines; to survive under the conditions of the stomach and the intestines, i.e. to survive under the conditions of acidic pH in the stomach and to be resistant to the action of bile [12]; to be able to reproduce in the gastro-intestinal tract; to suppress the pathogenic and toxigenic microorganisms [5, 6] and to push them out of the biological niche utilizing advantageously the substrate; to allow industrial fermentation; to possess antimicrobial activity against conditionally pathogenic, carcinogenic and pathogenic microorganisms; to produce antimicrobial substances; to modulate the immune response; to be safe for clinical and nutritional uses [14].

The purpose of the present work was to investigate some probiotic properties of *Lactobacillus plantarum* BG24 (isolated from naturally fermented cereal drink - boza) - resistance to different values of pH and enzymes (pH=2 + pepsin, pH=4.5 + pancreatin and pH=7 + pancreatin), different concentrations of bile salts, and the profile of its antibiotic resistance.

MATERIALS AND METHODS

Media

MRS-broth medium. Composition (g/dm³): peptone from casein - 10; yeast extract - 4; meat extract - 8; glucose - 20; K₂HPO₄ - 2; sodium acetate - 5; diammonium citrate - 2;

MgSO₄ - 0.2; MnSO₄ - 0.04; Tween 80-1 ml; pH = 6.5. The medium was sterilized for 15 minutes at 121°C.

LAPTg10-agar. Composition (g/dm³): peptone - 15; yeast extract - 10; tryptone - 10; glucose - 10. pH was adjusted to 6.6 - 6.8, and Tween 80 was added - 1cm³/dm³, agar - 1.5%. The medium was sterilized for 20 minutes at 121°C.

Determination of the survival at different values of pH and in the presence of pepsin or pancreatin [3]

Fresh 24-hour culture of studied strain was centrifuged for 15 min at 5000xg. The resulting biomass precipitate was washed twice with PBS-buffer and resuspended to the original volume in PBS-buffer. 0.2 cm³ of the cell suspension was incubated with 5 cm³ of the buffer solution with pH = 2, containing 0.5% NaCl and pepsin (with a concentration of 3.2 g/dm³) (Sigma, 2,500-3,500 U/mg protein) and a buffer solution with a pH=8, containing 0.5% NaCl and pancreatin (with a concentration of 1 g/dm³) (Sigma, 2,500-3,500 U / mg protein) at 37°C for 24 hours. At the 0th, 2nd, 4th and 24th hour an aliquot for determination of the number of viable lactobacilli cells (cfu/cm³) was taken.

Determination of the survival at different concentrations of bile salts in the medium [4]

MRS-broth medium with different concentrations of bile salts – 0%, 0.15%, 0.3%, 0.6% and 1% - was inoculated with 4% inoculum of the 24-hour culture of the tested strain. During culturing for 24 hours at 37°C aliquots for determination of the number of viable lactobacilli cells (cfu/cm³) were taken at the 0th, 2nd, 4th, 6th, 8th and 24th hour.

Determination of the antibiotic resistance [5]

The antibiotic resistance profile was determined by the disc diffusion method of Bauer, Kirby et al., (1966). Fresh 24 hour culture of the studied strain was used to inoculate Petri dishes with MRS-agar. Standard discs impregnated with antibiotic were placed in the Petri dishes. The dishes were incubated for 48 h at 37°C. The diameter (in mm) of the sterile zones formed around each of the antibiotic discs was recorded taking into account the following indications: R - resistant ($d_{zone} < 8$ mm), SR - intermediately sensitive ($d_{zone} = 8-16$ mm), S-sensitive ($d_{zone} > 16$ mm).

RESULTS AND DISCUSSION

In a series of experiments the resistance of the cells of *L. plantarum* BG24 under the model conditions of the gastro - intestinal tract was examined.

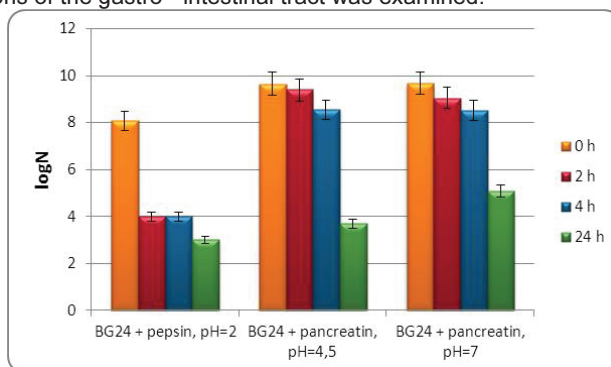


Fig. 1. Survival of cells of *Lactobacillus plantarum* BG24 under the conditions of acidic pH (pH = 2) + pepsin, pH = 4.5 + pancreatin and pH = 7 + pancreatin.

For the residence time of food in the stomach - 1.5 to 3h - in acidic environment (pH = 2 + pepsin) the number of viable cells of *Lactobacillus plantarum* BG24 was reduced by 5 logN. The strain was less sensitive to pH = 4,5 + pancreatin. The reduction of the concentration of viable cells by the 24th hour was 4 logN. At pH = 7 + pancreatin the reduction was 6 logN (Fig. 1).

In the cultivating of *Lactobacillus plantarum* BG24 in MRS-medium containing 0% bile salts over 10^{14} cfu/cm³ were accumulated by the 24th hour. The concentration of viable cells started decreasing from the first hours of incubation at all concentrations of bile salts. For 24 hours the degree of reduction was smaller at the lower concentrations of bile salts in the medium (0.15%, 0.3%) than at the higher concentrations of bile salts in the medium (0.6% and 1%), the values being 2logN and 3logN, respectively. Despite the high concentration of bile salts in the medium, the strain remains high titre of active cells(Fig. 2)

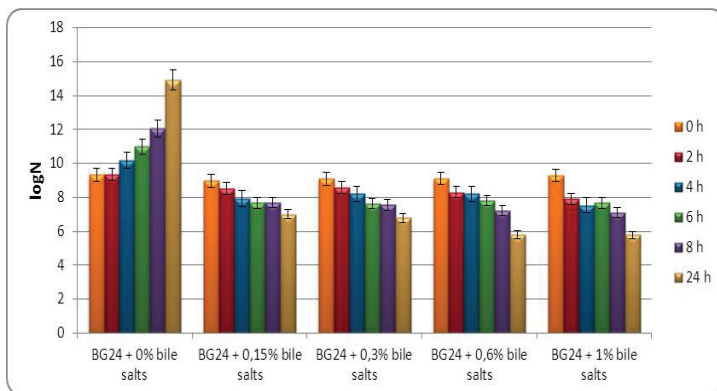


Fig. 2. Survival of the cells of *Lactobacillus plantarum* BG24 at different concentrations of bile salts in the medium.

The sensitivity of *Lactobacillus plantarum* BG24 was tested against 20 of the most frequently used in medical practice antibiotics with different mechanisms of action. The results of the studies carried out by the agar diffusion method of Bauer, Kirby et al., 1966, for 24 hours are summarized in Table 1.

Lactobacillus plantarum BG24 is resistant to all antibiotics from the group of the inhibitors of the synthesis of the cell walls with the exception of amoxicillin. From the group of the inhibitors of the protein synthesis the strain is sensitive to gentamicin, amikacin, erythromycin, rifampin and chloramphenicol. It has intermediate resistance to tetracycline and resistant to the other antibiotics of that group. The strain is resistant to both antibiotics - ciprofloxacin and nalidixic acid - from the group of the inhibitors of DNA synthesis and/or cell differentiation (Table 1).

Table. 1. Antibiotic resistance of *Lactobacillus plantarum* BG24

Mechanism of action	#	Antibiotic		Concentration	<i>L. plantarum</i> BG24
Inhibitors of the synthesis of the cell walls	1	Penicillin	P	10 E/disc	R
	2	Ampicillin	A	10 µg/disc	R
	3	Cefamandole	Cm	30 µg/disc	R
	4	Vancomycin	V	30 µg/disc	R
	5	Piperacillin	Pi	100 µg/disc	R
	6	Amoxicillin	Ax	25 µg/disc	S
	7	Azlocillin	Az	75 µg/disc	R
	8	Oxacillin	O	1 µg/disc	R
Inhibitors of the protein synthesis	9	Doxycycline	D	30 µg/disc	R
	10	Gentamicin	G	10 µg/disc	S
	11	Kanamycin	K	30 µg/disc	R
	12	Lincomycin	L	15 µg/disc	R
	13	Tobramycin	Tb	10 µg/disc	R
	14	Amikacin	Am	30 µg/disc	S
	15	Tetracycline	T	30 µg/disc	SR
	16	Erythromycin	E	15 µg/disc	S
	17	Rifampin	R	5 µg/disc	S
	18	Chloramphenicol	C	30 µg/disc	S
Inhibitors of DNA synthesis and/or cell division	19	Ciprofloxacin	Cp	5 µg/disc	R
	20	Nalidixic acid	Nx	30 µg/disc	R

CONCLUSION

Lactobacillus plantarum BG24 retained high concentration of viable cells in the in vitro conditions of the gastrointestinal tract - low pH values (pH = 2) + pepsin; (pH=4.5) + pancreatin; (pH=7) + pancreatin and at different concentrations of bile salts and was resistant to the majority of antibiotics applied in clinical practice, which makes it suitable for inclusion in the composition of probiotics and starters for the preparation of functional cereal foods.

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This paper has been reviewed