PROBIOTIC PROPERTIES OF *LACTOBACILLUS ACIDOPHILUS* Z10, ISOLATED FROM NATURALLY FERMENTED SOURDOUGH

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Received 6 August 2012, accepted 7 September 2012

Abstract: *The strains included in the composition of probiotic preparations have to possess a number of probiotic properties including the ability to survive and reproduce in the conditions in the gastrointestinal tract and antibiotic resistance. The resistance of Lactobacillus acidophilus Z10, isolated from naturally fermented sourdough, to gastric and pancreatic juices is examined by incubation of the strain in MRS-broth medium with pH=2, containing pepsin; pH=4.5, containing pancreatin and pH=7, containing pancreatin, as well as incubation in MRS-broth medium, containing different concentrations of bile salts. The profile of antibiotic resistance is determined by the disc diffusion method. The cells of Lactobacillus acidophilus Z10 are resistant to the model conditions of the gastrointestinal tract. The profile of antibiotic resistance of Lactobacillus acidophilus Z10 against 20 of the most commonly applied antibiotics in medical practice is examined and the strain is resistant to most of them. The resistance of the strain to most of the antibiotics included in the study together with its resistance to the model conditions of the gastrointestinal tract makes Lactobacillus acidophilus Z10 a potentially probiotic strain.*

Key words: *Lactobacillus, probiotic, gastric juice, pancreatic juice, bile salts, antibiotic resistance*

1. Introduction

Probiotics are live microorganisms that confer a beneficial effect on the host when administered in proper amounts [1, 2]. Their beneficial effects on gastrointestinal infections, the reduction of serum cholesterol, the protection of the immune system, anti-cancer properties, antimutagenic action, anti-diarrheal properties, the improvement in inflammatory bowel disease and suppression of *Helicobacter pylori* infection, Crohn's disease, restoration of the microflora in the stomach and the intestines after antibiotic treatment, etc. are proven by addition of selected strains to food products [3, 4, 5, 6].

Lactobacilli and bifidobacteria are normal components of the healthy human intestinal microflora. They are included in the composition of probiotics and probiotic foods because of their proven health effects on the body [7, 8, 9]. They are the main organisms that maintain the balance of the gastrointestinal microflora [10].

Not all strains of lactobacilli and bifidobacteria can be used as components of probiotics and probiotic foods, but only those that are of human origin, non-pathogenic, resistant to gastric acid, bile and to the antibiotics, administered in medical practice; they should also have the potential to adhere to the gut epithelial tissue and produce antimicrobial substances; they should allow the

conduction of technological processes, in which high concentrations of viable cells are obtained as well as to allow industrial cultivation, encapsulation and freeze-drying and they should remain active during storage [11, 12]. This requires the mandatory selection of strains of the genera *Lactobacillus* and *Bifidobacterium* with probiotic properties. Moreover, the concentration of viable cells of microorganisms in the composition of probiotics should exceed 1 million per gram [13] in order for the preparation to exhibit a therapeutic and prophylactic effect. The survival of probiotic bacteria in the gastrointestinal tract, and their translocational and colonizational properties and the destruction of their active components are essential for the realization of their preventive role.

Different probiotic strains react differently in different parts of the gastrointestinal tract - some strains are killed very quickly in the stomach, while others pass through the entire gastrointestinal tract at high concentrations [14, 15, 16, 17, 18, 19, 20, 21, 22].

The purpose of the present paper is to examine some of the probiotic properties of the strain *Lactobacillus acidophilus* Z10 isolated from naturally fermented sourdough: determination of the profile of antibiotic resistance, determination of the resistance of the strain to the model conditions of gastric and pancreatic juice, as well as to elevated concentrations of bile salts.

2. Materials and methods

Microorganisms

The studied strain *Lacobacillus acidophilus* Z10 is isolated from naturally fermented sourdough.

Nutrient media

MRS – broth medium (medium of De Man, Rogosa & Sharpe).

Composition (g/dm^3): peptone from casein - 10 g; yeast extract - 4 g; meat extract - 8 g; glucose - 20 g; K_2HPO_4 - 2 g; sodium acetate - 5 g; diammonium citrate - 2 g; $MgSO_4$ - 0.2 g; $MnSO_4$ - 0.04 g; Tween 80-1 ml; pH = 6.5. The medium is sterilized for 15 min at 118ºC.

MRS – agar medium.

Composition (g/dm^3): MRS - broth +2% agar. The medium is sterilized for 15 min at 118ºC.

LAPTg10-agar medium.

Composition (g/dm^3): LAPTg10-broth medium + 2% agar. The medium is sterilized for 20 minutes at 121ºC.

Saline.

Composition (g/dm^3): NaCl - 5 g; distilled water - 1l. Sterilization for 20 min at 121ºC.

Cultivation and storage of the studied microorganism

The studied strain is cultivated in a liquid medium (MRS-broth) and on agar medium (MRS-agar) at 37ºC. It is isolated from a single colony and is cultivated in MRS-broth medium for 24 hours. The strain is stored as a stock-culture in MRS-broth with 20% v/v glycerol at -20ºC.

Determination of the profile of antibiotic resistance

The profile of antibiotic resistance is determined by the disk diffusion method of Bauer, Kirby et al. [23]. Fresh 24-hour culture of the tested strain is used to inoculate the plates with LAPTg10-agar. Standard discs impregnated with antibiotics are placed in the plates. The plates are incubated for 48 hours at optimum temperature. The diameters (in mm) of the sterile zones formed around each of the antibiotic discs are recorded. Then they are subjected to the following designations: R - resistant (zone < 8 mm),

SR - intermediately sensitive (zone 8-16 mm), S - sensitive (zone > 16 mm).

***Determination of the resistance to low pH in the presence of pepsin and to weakly alkaline pH in the presence of pancreatin* [24]**

Fresh 24 - hour culture of the studied strain is centrifuged for 15 min at 5,000 x g. The resulting sludge biomass is washed twice with PBS - buffer and resuspended to the initial volume in PBS - buffer. 0.2 cm^3 of the cell suspension are incubated with 5 cm^3 buffer solution with pH = 2 containing 0,5% NaCl and pepsin (at a concentration of 3.2 g/dm^3) (Sigma, 2,500 - 3,500 U / mg protein), buffer with pH = 4,5 + pancreatin and buffer with pH = 7 + pancreatin at a suitable temperature for the studied strain (37°C) for 24h. At the 0, the 2^{nd}, the 4^{th} and the 24^{th} hour aliquots for the determination of the number of viable cells are taken (cfu/cm^3).

***Determination of the tolerance to bile salts (method modified by Denkova Z., 2005* [22])**

Fresh 24 - hour culture of the studied strain is centrifuged for 15 min at 5,000 x g. The resulting sludge biomass is washed twice with PBS - buffer and resuspended to the initial volume in PBS - buffer. 0.2 cm^3 of the cell suspension are incubated with 5 cm^3 of the MRS- broth medium with different concentrations of bile salts - 0%, 0.15%, 0.3%, 0.6% and 1% - for 24h at the optimum temperature for the strain (37°C), and aliquots for the determination of the number of viable cells (cfu/cm^3) at the 0, the 2^{nd}, the 4^{th}, the 6^{th}, the 8^{th} and the 24^{th} hour are taken.

3. Results and discussion

A series of tests are conducted in order to determine the probiotic potential of the strain *Lactobacillus acidophilus* Z10 with optimum temperature 37°C.

In vitro determination of the ability of Lactobacillus acidophilus Z10 to survive in conditions simulating the various departments of the gastrointestinal tract

The resistance of the cells of *Lactobacillus acidophilus* Z10 in model conditions of the gastro - intestinal tract - pH = 2 + pepsin, pH = 4,5 + pancreatin and pH = 7 + pancreatin is examined. In a parallel experiment the tolerance of this strain to different concentrations of bile salts is tested. The results of the experimental studies are presented on Fig. 1 and Fig. 2.

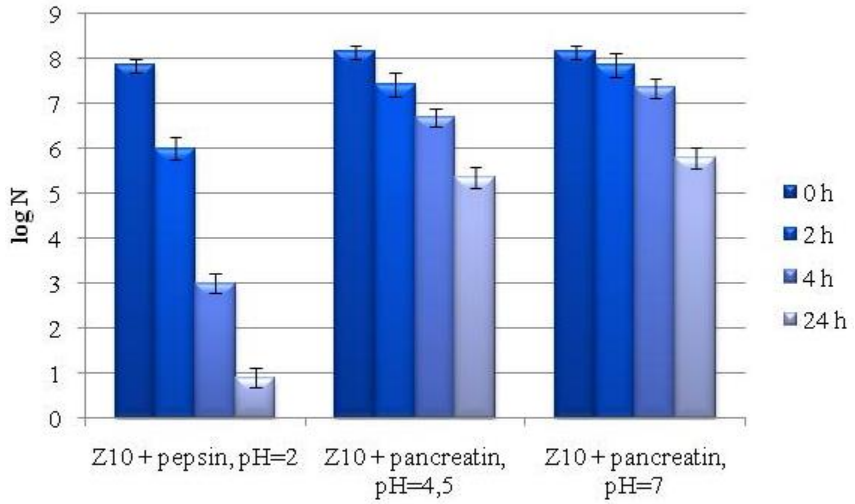

Fig. 1. Survival of the cells of the strain Lactobacillus acidophilus Z10 in acidic pH (pH = 2) + pepsin, pH = 4,5 + pancreatin and pH = 7 + pancreatin.

Higher sensitivity to low pH = 2 + pepsin than to pH = 4,5 + pancreatin and pH = 7 + pancreatin is observed (Fig. 1). By the 24th hour of cultivation of the strain at pH=2 + pepsin the concentration of viable cells decreases by 7logN. For 24 - hour incubation at pH = 4,5 + pancreatin the reduction in the number of viable cells is 2.8 logN, while at pH = 7 + pancreatin – 2.4 logN.

Another important factor that influences the survival of probiotic strains in the intestinal tract are bile salts. It is known that about three hours after ingestion of food the concentration of bile salts in the small intestine reaches about 0.3%. This requires a study on the influence of different concentrations of bile salts on the development of *Lactobacillus acidophilus* Z10. It is conducted by incubation of *Lactobacillus acidophilus* Z10 in MRS-broth medium with different concentrations of bile salts, 0%, 0.15%, 0.3%, 0.6% and 1% for 24 hours of incubation (Fig. 2).

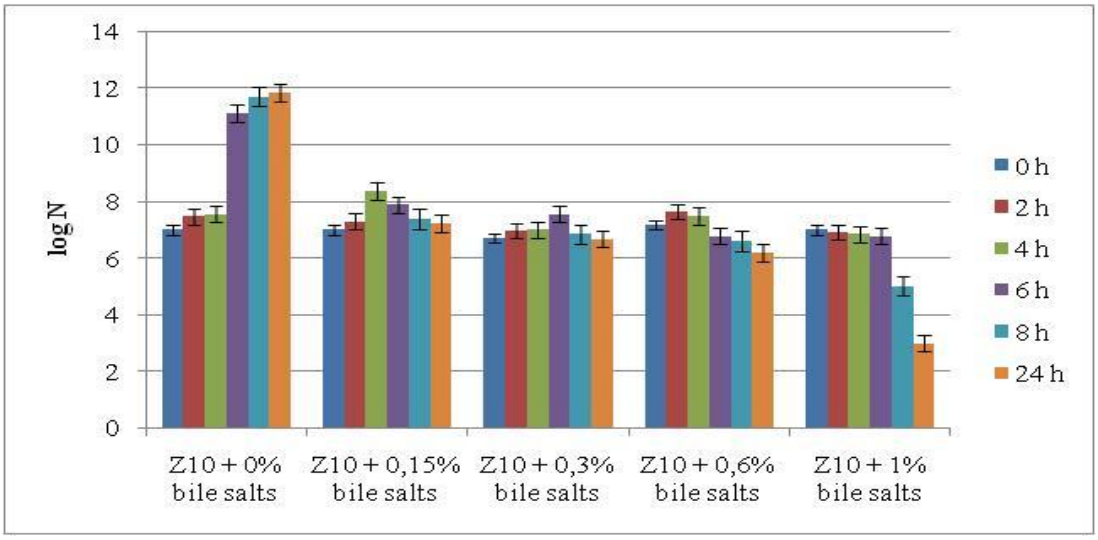

Fig. 2. Survival of the cells of Lactobacillus acidophilus Z10 at different concentrations of bile salts.

The experimental data presented on Fig. 2 show that in the first four hours of the incubation of *Lactobacillus acidophilus* Z10 in the presence of bile salts the number of viable cells is retained. This retention is a result of the development of naturally sustainable branches, which is consistent with the test of Luria and Delbruk. After the fourth hour, a reduction of the number of viable cells in varying degrees depending on the concentration of bile salts is observed. 1.10^3cfu/cm^3 active cells are defined at 1% bile salts in the medium at the 24th hour.

Antibiotic resistance of Lactobacillus acidophilus Z10

20 of the most commonly used antibiotics in medical practice antibiotics with different mechanisms of action are selected and the sensitivity of *Lactobacillus acidophilus* Z10 towards them is tested. The results of the studies using the agar diffusion method [23] for 24 hours are summarized in Table. 1.

Lactobacillus acidophilus Z10 is sensitive to three of the antibiotics from the group of the inhibitors of the synthesis of the cell walls, azlocillin, piperacillin and vancomycin, and is resistant to the other 5 antibiotics in this group. The strain is

resistant to 4 out of 10 antibiotics that inhibit protein synthesis, but it demonstrates sensitivity towards lincomycin, chloramphenicol and erythromycin, and intermediate sensitivity towards tetracycline, doxycycline and amikacin. *Lactobacillus acidophilus* Z10 is resistant to the antibiotics inhibiting the synthesis of DNA and/or cell division (Table 1). The strain is resistant to most of the antibiotics included in the study.

Table 1.
Antibiotic resistance of Lactobacillus acidophilus Z10
Legend: R-resistant, SR – intermediate sensitivity (zone 7-16 mm), S - sensitive (zone> 16 mm)

#	Mechanism of action	Antibiotic		Concentration	***Lactobacillus acidophilus*** Z10
1	Inhibitor of the synthesis of the cell walls	Penicillin	P	10 E/disc	R
2		Azlocillin	Az	75 µg/disc	S
3		Piperacillin	P	100 µg/disc	S
4		Ampicillin	A	10 µg/disc	R
5		Oxacillin	O	1 µg/disc	R
6		Amoxicillin	Ax	25 µg/disc	R
7		Vancomycin	V	30 µg/disc	S
8		Cefamandole	Cm	30 µg/disc	R
9	Inhibitor of the protein synthesis	Tetracycline	T	30 µg/disc	SR
10		Doxycycline	D	30 µg/disc	SR
11		Gentamicin	G	10 µg/disc	R
12		Kanamycin	K	30 µg/disc	R
13		Tobramycin	Tb	10 µg/disc	R
14		Amikacin	Am	30 µg/disc	SR
15		Rifampin	R	5 µg/disc	R
16		Lincomycin	L	15 µg/disc	S
17		Chloramphenicol	C	30 µg/disc	S
18		Erythromycin	E	15 µg/disc	S
19	Inhibitor of the synthesis of DNA and/or cell division	Nalidixic acid	Nx	30 µg/disc	R
20		Ciprofloxacin	Cp	5 µg/disc	R

1. Conclusion

Lactobacillus acidophilus Z10 has the ability to survive in the model conditions of the gastro - intestinal tract and is resistant to most of the antibiotics applied in medical practice. Thus, it can be defined as a potential probiotic culture.

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