# PROBIOTIC PROPERTIES OF LACTOBACILLUS ACIDOPHILUS A2 OF HUMAN ORIGIN

***[1]Denkova Rositsa, [2]Dimbareva Donka, [2]Denkova Zapryana**

[1]Sofia University "St. Kliment Ohridski" – Sofia, Bulgaria
[2]University for Food Technologies – Plovdiv, Bulgaria

***Denkova Rositsa Stefanova, rositsa_denkova@mail.bg**

**Abstract:** The strain *Lactobacillus acidophilus* A2 is of human origin. The resistance of its cells in model conditions of digestion - low values of pH (pH = 2) + pepsin, pH = 4.5 + pancreatin and pH = 7 + pancreatin and to different concentrations of bile salts is determined. It has been shown that the cells of *Lactobacillus acidophilus* A2 are resistant to the conditions of the gastrointestinal tract.
The profile of antibiotic resistance of *Lactobacillus acidophilus* A2 against 20 of the most commonly applied antibiotics in medical practice is examined and the strain is resistant to most of them, which together with the resistance of *Lactobacillus acidophilus* A2 to the model conditions of the gastrointestinal tract makes *Lactobacillus acidophilus* A2 a potentially probiotic strain.
**Key words:** Lactobacillus, probiotic, gastric juice, pancreatic juice, bile salts

## 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* A2 of human origin: 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.

**Materials and methods**

1. Microorganisms

The studied strain *Lacobacillus acidophilus* A2 is of human origin.

2. Media:

2.1. 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.

2.2. MRS – agar medium.

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

2.3. LAPTg10-agar medium.

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

2.4. Saline.

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

3. Cultivation and storage of microorganisms studied

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.

4. Determination of the profile of antibiotic resistance

The profile of antibiotic resistance is determined by the disk diffusion method of Bauer, Kirby et al. 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).

4.2. Determination of the resistance to low pH in the presence of pepsin and to weakly alkaline pH in the presence of pancreatin (Charteris WP et al., 1998)

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 2nd, the 4th and the 24th hour aliquots for the determination of the number of viable cells are taken (cfu/cm$^3$).

4.3. Determining the tolerance to bile salts (method modified by Denkova Z., 2005)

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 2nd, the 4th, the 6th, the 8th and the 24th hour are taken.

**Results and discussion**

A series of tests are conducted in order to determine the probiotic potential of the strain *Lactobacillus acidophilus* A2 with optimum temperature 37°C.

1. *In vitro* determination of the ability of *Lactobacillus acidophilus* A2 to survive in conditions simulating the various departments of the gastrointestinal tract

The resistance of the cells of *Lactobacillus acidophilus* A2 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 high concentrations of bile salts is tested. The results of the experimental studies are presented on Fig. 1 and Fig. 2.

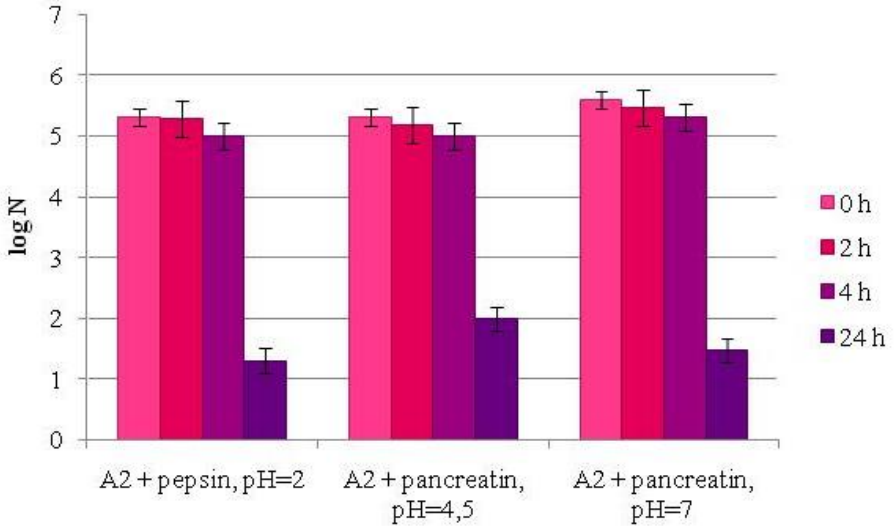


***Fig. 1.*** Survival of the cells of the strain Lactobacillus acidophilus A2 in acidic pH (pH = 2) + pepsin, pH = 4,5 + pancreatin and pH = 7 + pancreatin.

Higher sensitivity to low pH = 2 + pepsin than to pH = 4,5 + pancreatin and pH = 7 + pancreatin (Fig. 1) is observed. By the 24th hour of cultivation of the strain in acidic environment, the concentration of viable cells decreases by 75%. For 24 - hour incubation

at pH = 4,5 + pancreatin the reduction in the number of viable cells is 3.3 log units, while at pH = 7 + pancreatin - 4.1 log units.

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 study of the influence of different concentrations of bile salts on the development of *Lactobacillus acidophilus* A2 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).

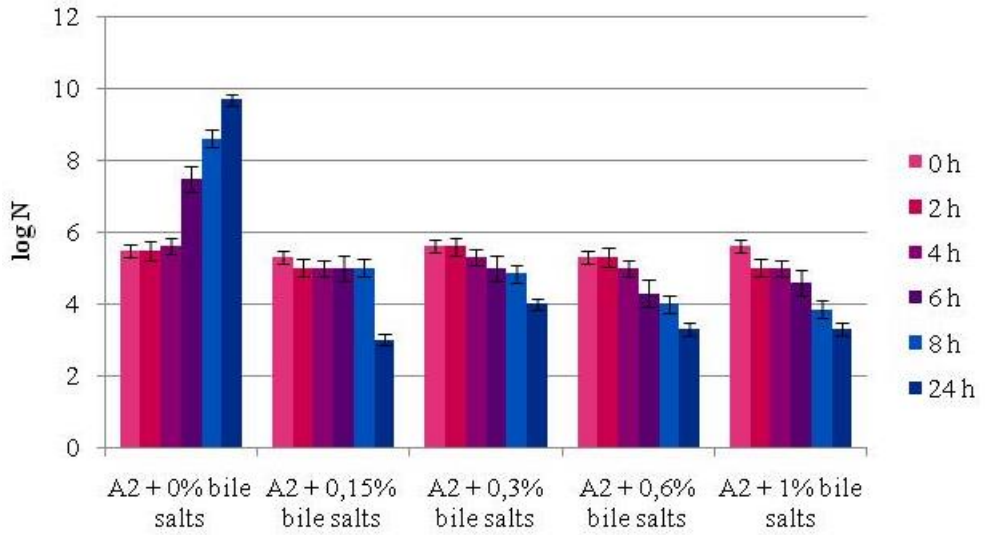


***Fig. 2.*** Survival of the cells of Lactobacillus acidophilus A2 at different concentrations of bile salts.

The experimental data presented on Fig. 2 show that in the first four hours of the incubation of *Lactobacillus acidophilus* A2 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. $2.10^3$cfu/cm$^3$ active cells are defined at 1% bile salts in the medium at the 24$^{th}$ hour.

Antibiotic susceptibility of *Lactobacillus acidophilus* A2

20 antibiotics with different mechanisms of action that are some of the most commonly used antibiotics in medical practice are selected and the sensitivity of *Lactobacillus acidophilus* A2 is tested. The results of the studies using the agar diffusion method by Bauer, Kirby et al., (1966) for 24 h are summarized in Table. 1.

*Lactobacillus acidophilus* A2 is sensitive to lincomycin, but resistant to most of the antibiotics included in the study except azlotsilin and amoxicillin, towards which it demonstrates intermediate susceptibility (Table 1).

***Table 1.*** Antibiotic susceptibility of Lactobacillus acidophilus A2

| # | Mechanism of action | Antibiotic | | Concentration | ***Lactobacillus acidophilus*** A2 |
|---|---|---|---|---|---|
| 1 | Inhibitor of the synthesis of the cell walls | Penicillin | P | 10 E/диск | R |
| 2 | | Azlocillin | Az | 75 μg/диск | SR |
| 3 | | Piperacillin | P | 100 μg/диск | R |
| 4 | | Ampicillin | A | 10 μg/диск | R |
| 5 | | Oxacillin | O | 1 μg/диск | R |
| 6 | | Amoxicillin | Ax | 25 μg/диск | SR |
| 7 | | Vancomycin | V | 30 μg/диск | R |
| 8 | | Cefamandole | Cm | 30 μg/диск | R |
| 9 | Inhibitor of the protein synthesis | Tetracycline | T | 30 μg/диск | R |
| 10 | | Doxycycline | D | 30 μg/диск | R |
| 11 | | Gentamicin | G | 10 μg/диск | R |
| 12 | | Kanamycin | K | 30 μg/диск | R |
| 13 | | Tobramycin | Tb | 10 μg/диск | R |
| 14 | | Amikacin | Am | 30 μg/диск | R |
| 15 | | Rifampin | R | 5 μg/диск | R |
| 16 | | Lincomycin | L | 15 μg/диск | S |
| 17 | | Chloramphenicol | C | 30 μg/диск | R |
| 18 | | Erythromycin | E | 15 μg/диск | R |
| 19 | Inhibitor of the synthesis of the synthesis of DNA and/or cell division | Nalidixic acid | Nx | 30 μg/диск | R |
| 20 | | Ciprofloxacin | Cp | 5 μg/диск | R |

Legend: R-resistant, SR – intermediate sensitivity (zone 7-16 mm), S - sensitive (zone> 16 mm)

## Conclusion

*Lactobacillus acidophilus* A2 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.

## References

[1] Kalliomaki M., Salminen S., Arvilommi H., Kero P., Koskinen P., Isolauri E. (2001). Probiotics in primary prevention of atopic disease: a randomised placebocontrolled trial. Lancet 357: 1076-1079.

[2] Brown A. C., Valiere A. (2004). Probiotics and medical nutrition therapy. Nutr. Clin. Care 7: 56-68.

[3] Agerholm-Larsen L., Raben A., Haulrik N., Hansen A. S., Manders M., Astrup A. (2000). Effect of 8 week intake of probiotic milk products on risk factors for cardiovascular diseases. Eur. J. Clin. Nutr. 54: 288-297.

[4] Nomoto K. (2005). Review prevention of infections by probiotics. J. Biosci. Bioeng.100: 583-592.

[5] Imasse K., Tanaka A., Tokunaga K., Sugano H., Ishida H., Takahashi S. (2007). Lactobacillus reuteri tablets suppress Helicobacter pylori infectionda doubleblind

randomised placebo-controlled cross-over clinical study Kansenshogaku zasshi. J. Jpn. Assoc. Infect. Dis. 81: 387-393.
[6] Shah N. P. (2007). Functional cultures and health benefits. Int. Dairy J. 17: 1262-1277.
[7] Hirayama K., Rafter J. (2000). The role of probiotic bacteria in cancer prevention. Microbes Infect. 2: 681-686.
[8] Isolauri E. (2001). Probiotics in human disease. American Journal of Clinical Nutrition, 73 (6): 1142S-1146.
[9] Marteau P. R., de Vrese M., Cellier C. J., Schrezenmeir J. (2001). Protection from gastrointestinal diseases with the use of probiotics. American Journal of Clinical Nutrition 73 (Suppl. 2): 430S-436S.
[10] Rybka S., Kailasapathy K. (1995). The survival of culture bacteria in fresh and freeze-dried AB yoghurts. The Australian Journal of Dairy Technology 50 (2): 51-57.
[11] Mitsuoka T. (1999). The human gastrointestinal tract. In: Wood BJB, editor. The lactic acid bacteria. vol.1, Gaithersburg, MD, USA: Aspen Publishers Inc.: 69-114 p.
[12] Kirtzalidou E., Pramateftaki P., Kotsou M., Kyriacou A. (2011). Screening for lactobacilli with probiotic properties in the infant gut microflora. Anaerobe 17: 440-443.
[13] Donald J., Brown D. (1993) Probiotics and the intestinal ecosystem. Let's live, November, 45-47.
[14] Pochart P., Mavtean P., Bouhnik Y., Goderel I., Bourlioux P., Rambrand, J. C., (1992). Survival of bififdobacteria ingested via fermented milk during their passage trough the human small intestine: an in vivo study using intestinal perfusion. Am. J. Clin. Nutr. , 55, 78-80.
[15] Nielsen E. M., Schlundt J., Gunvig A., Jacobsen B.L. (1994). Epithelial mucus and lumen subpopulations of Escherichia coli in the large intestine of conventional and gnotobiotic rats. Microbial. Ecol. Health Dis. 7, 263-273.
[16] Alander M., Korpela R., Saxelin M., Vilponen-Salmela T., Mattila-Sandholten T., von Wright A. (1997). Recovery of *Lactobacillus rhamnosus* GG from human colonic biopsies. Lett. Appl. Microbiol. 24, 361-364.
[17] Donohue D. C., Salminen S. (1996). Safety assessment of probiotic bacteria. Asia Pac. J. Clin. Nutr. 5, 25-28.
[18] Donohue D. C., Salminen S., Marteau P. (1998). Safety of probiotic bacteria. In: Salminen S., A.von Wright (Eds.), Lactic acid bacteria. "Marcel Dekker" INC, New York, 369-384.
[19] Adams M. R. (1999). Safety of industrial lactic acid bacteria. J. Biotechnol., 68, 171-178.
[20] Saarela M., Mogensen G. (2000). Probiotic bacteria: Safety, functional and technological properties. Journal of biotechnology, 84, 197-215.
[21] Nikolova, D. (2010). "Probiotichni i biotehnologichni harakteristiki na shtamove ot rod *Lactobacillus*" PhD thesis.
[22] Denkova Z. (2005). "Poluchavane i prilojenie na probiotici" D.Sc. thesis.