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THE EFFECT OF HIGH PRESSURE ON SELECTED THREE STRAINS OF *LACTOBACILLUS ACIDOPHILUS*

Aleksandra Grześkiewicz, Agnieszka Jankowska, Krystyna Wiśniewska, Arnold Reps

Chair of Food Biotechnology
University of Warmia and Mazury in Olsztyn

K e y w o r d s: *Lactobacillus acidophilus*, pressurization, antibacterial activity, acidifying activity.

A b s t r a c t

The experiment was aimed at determining the influence of pressures between 30 and 150 MPa on selected three strains of *Lactobacillus acidophilus* on bacterial count; the antibacterial activity and acidifying activity. Cultures of the following strains: *Lactobacillus acidophilus LA-5*, *Lactobacillus acidophilus T132/2* and *Lactobacillus acidophilus T294*, in milk and liquid MRS medium were subjected to a pressure treatment of 30, 60, 90, 120 and 150 MPa for 1 minute. Immediately after pressurization and after inoculation, the cultures were determined for survivability, active acidity (pH) and antibacterial activity. The study demonstrated that pressure did not exert any significant effect on the survivability of the strains examined. An increase in the acidifying activity of *Lactobacillus acidophilus T294* strain was observed after pressurization in a culture run on milk. The antibacterial activity was found to depend on the culture medium. Pressurization had a little effect on the improvement of antibacterial properties of the examined strains of *Lactobacillus acidophilus*.

WPŁYW WYSOKICH CIŚNIEŃ NA WYBRANE TRZY SZCZEPY *LACTOBACILLUS ACIDOPHILUS*

Aleksandra Grześkiewicz, Agnieszka Jankowska, Krystyna Wiśniewska, Arnold Reps

Katedra Biotechnologii Żywności
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: *Lactobacillus acidophilus*, presuryzacja, aktywność antybakteryjna.

Address: Aleksandra Grześkiewicz, University of Warmia and Mazury, ul. Heweliusza 1, 10-724 Olsztyn, Poland, phone: +48 (89) 523 32 33, e-mail: aleksandra.grzeskiewicz@uwm.edu.pl

Abstrakt

Celem doświadczenia było określenie wpływu ciśnień 30–150 MPa na przeżywalność, aktywność kwaszącą i aktywność antybakteryjną szczepów: *Lactobacillus acidophilus LA-5*, *Lactobacillus acidophilus T132/2* i *Lactobacillus acidophilus T294*. Hodowle szczepów na mleku i na podłożu płynnym MRS poddano działaniu ciśnienia 30, 60, 90, 120 i 150 MPa przez 1 minutę. Bezpośrednio po presuryzacji oraz po przeszczepieniu oznaczano przeżywalność, kwasowość czynną (pH) i aktywność antybakteryjną. Stwierdzono, że ciśnienie nie wpływa znacząco na przeżywalność badanych szczepów. Najniższą aktywność kwaszącą wykazywały bakterie *Lactobacillus acidophilus T294* w hodowli na mleku. Stwierdzono niewielki wzrost aktywności kwaszącej badanych szczepów po presuryzacji w hodowlach na mleku. W hodowlach na podłożu płynnym MRS aktywność antybakteryjna badanych szczepów była wyższa niż w hodowlach na mleku. Presuryzacja, bez względu na stosowane ciśnienie, wpłynęła nieznacznie na poprawę właściwości antybakteryjnych badanych szczepów *Lactobacillus acidophilus*.

Introduction

Lactobacillus acidophilus is one of the major species of natural microflora colonizing the gastrointestinal tract of humans and animals. In breast-fed infants probiotic bacteria, like *Bifidobacterium sp.*, represent 99% of intestinal microflora. Strains of *Lactobacillus acidophilus* are characterized by a number of health-promoting properties, i.e. antibacterial activity against pathogenic microflora, enhancement of the digestion process of lactose, capability to assimilate cholesterol and support the immune system of humans. (JAKUBCZYK and KOSIKOWSKA 1996, OUWEHAND et al. 2002, PARVEZ et al. 2006).

Investigations carried out thus far have indicated that high pressure exerts a variety of effects on microorganisms depending not only on the species but also on the strain. The result of pressurization is affected by both the value of pressure, length of pressure treatment and the course of enzymatic reactions. Thus, pressurization is likely to influence the functioning and metabolism of microorganisms (REPS et al. 1994).

In the reported study, assays were conducted to examine the effect of pressurization at 30–150 MPa on survivability, antibacterial activity and acidifying activity of the following strains: *Lactobacillus acidophilus LA-5*, *Lactobacillus acidophilus T132/2*, *Lactobacillus acidophilus T294*.

Materials and Methods

Materials

The following strains were used in this study: *Lactobacillus acidophilus LA-5*, *Lactobacillus acidophilus T132/2* and *Lactobacillus acidophilus T294*.

These strains were cultured on sterile reconstituted skim-milk (10% d.m.) and on sterile liquid MRS medium (Merck) at pH 5.4. The inoculum averaged about 10^2 cfu cm^{-3}.

The course of the experiment

In duplicate cultures of *Lactobacillus acidophilus* (passage I) were poured into 10 cm^3 plastic containers and pressurized in a high-pressure generator (Unipress Equipment) using pressures of 30, 60, 90, 120 and 150 MPa for 1 minute at 18°C.

Immediately after pressure treatment, bacterial count, acidifying activity and antibacterial activity, assays were conducted in the non-pressurized and pressurized cultures.

Next, the pressurized and non-pressurized cultures were inoculated at 2% onto reconstituted skim-milk (10% d.m.) and sterile liquid MRS medium (pH 5.4), incubated at 37°C/20 h and then kept at a temperature of 4°C/12 h, thus passage II was obtained. The resultant cultures were subjected to the same assays as those of passage I.

Analytical methods

Bacterial count of *Lactobacillus acidophilus* was determined on MRS--Agar (Merck) medium (pH 5.4) using the plate method. Incubation was run at 37°C/72 h under anaerobic conditions.

The antibacterial activity was determined with a modified well method against 11 test strains: *Escherichia coli 366, Escherichia coli 345, Escherichia coli Ł1, Enterobacter cloacae, Enterobacter cloacae 10, Enterobacter cloacae 11, Enterobacter cloacae 17, Proteus 6H, Proteus dw, Klebsiella ssp. 499, Klebsiella pneumoniae B*. Agar broth medium (1.25%), previously inoculated with a liquid culture of a test strain was poured onto wells so that the number of cells in the medium reached 10^4–10^5 cfu cm^{-3}. After setting, wells 10 mm in diameter were cut in the medium. Next, 0.125 cm^3 of the examined material were introduced into each well and the medium was incubated at 37°C/16–24 h under aerobic conditions. Following the incubation, the size of growth inhibition zones of the test strains was measured and expressed in mm.

Active acidity was measured with an HI 221 pH-meter (HANNA instruments).

All the obtained results were subjected to statistical analysis.

Results and Discussion

Effect or pressurization on the survivability of the examined strains of *Lactobacillus acidophilus*

Pressure treatments at 30–150 MPa did not elicit any significant differences in the survivability of *Lactobacillus acidophilus* strains (Student's t-distribution, $\alpha = 1\%$). As compared to the control sample (non-pressurized), the number of bacteria in samples subjected to pressure treatment was lower by less than one logarithmic cycle (Figure 1–3) of the strains examined,

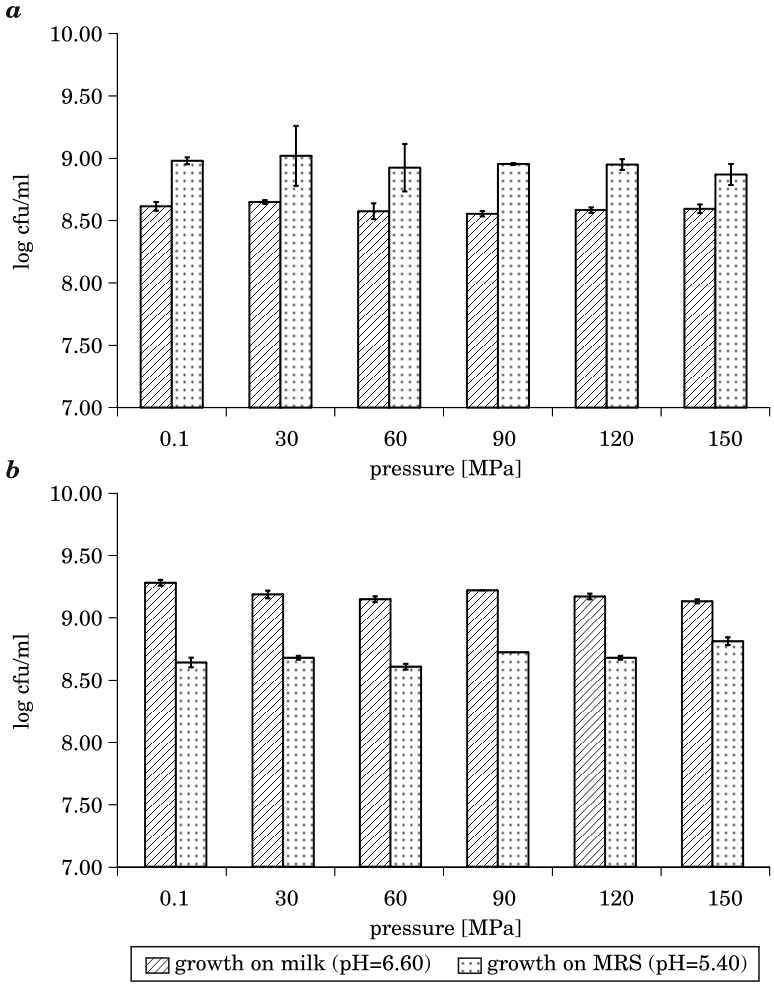

Fig. 1. Survivability of Lactobacillus acidophilus: *a* – *LA-5* – I passage, *b* – *LA-5* – II passage

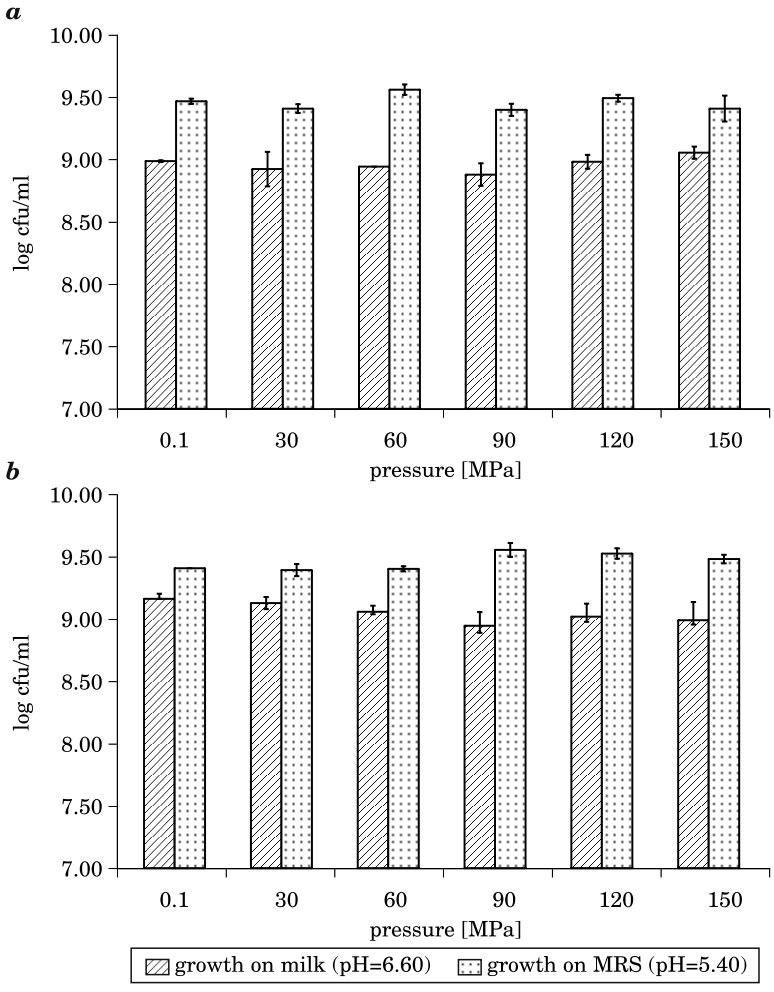

Fig. 2. Survivability of Lactobacillus acidophilus: *a* – 132/2 – I passage, *b* – 132/2 – II passage

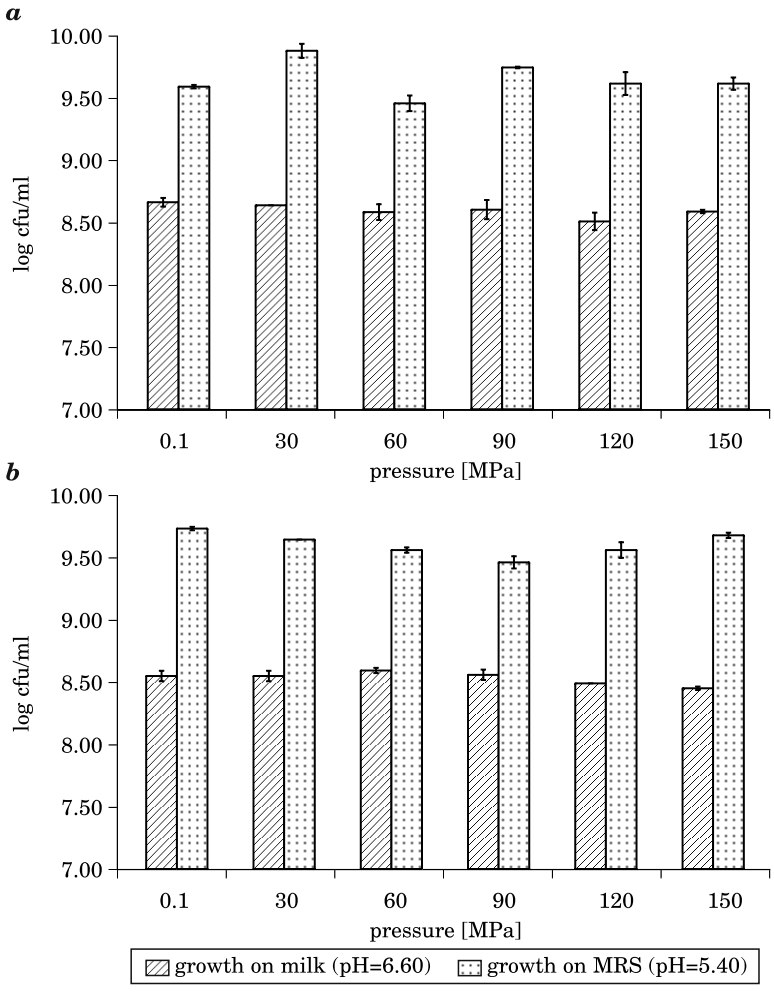

Fig. 3. Survivability of Lactobacillus acidophilus: *a* – 294 – I passage, *b* – 294 – II passage

the lowest resistance to the action of pressure was observed in the case of *Lactobacillus acidophilus* LA-5 strain.

The survivability of the examined strains of *Lactobacillus acidophilus* pressurized in the culture grown on the MRS medium was higher compared to those strains cultured on milk. No differences were found in the survivability between non-pressurized and pressurized strains in the passage II on milk and MRS medium.

Investigations by WOUTERS et al. (1998) demonstrated that the susceptibility of microorganisms to the action of high pressure depends on the reaction of medium. *Lactobacillus plantarum* grown on a medium at pH 5.0 subjected to

a pressure treatment of 250 MPa was found to be more resistant than in the culture run on a medium at pH 7.0.

LANCIOTTI et al. (2007) investigated the effect of high-pressure homogenization on the survivability of selected bacteria of the genus *Lactobacillus*. They did not observe any significant changes in the number of those bacteria after pressure treatments at 50, 100 and 150 MPa.

In passage II, the strains subjected to pressurization exhibited the same, and in some cases even higher survivability as compared to the non-pressurized strains.

Effect of pressurization on the acidifying activity of the examined strains of *Lactobacillus acidophilus*

The highest acidifying activity was demonstrated for the strain *Lactobacillus acidophilus LA-5*. A slight increase in the acidifying activity of the analyzed strains was found after pressurization in cultures run on milk (passage II) – Figure 4–6.

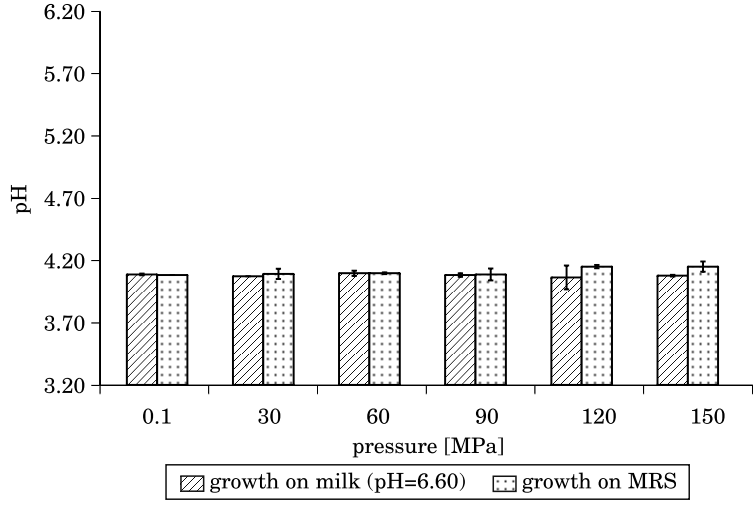

Fig. 4. Acidifying activity of *Lactobacillus acidophilus LA-5* – II passage

LIKEWISE, LANCIOTTI et al. (2007) observed an increase in the acidifying activity of selected strains of the genus *Lactobacillus* after a pressure treatment at 50 MPa. Additionally their study demonstrated that differences in that activity were strain-dependent.

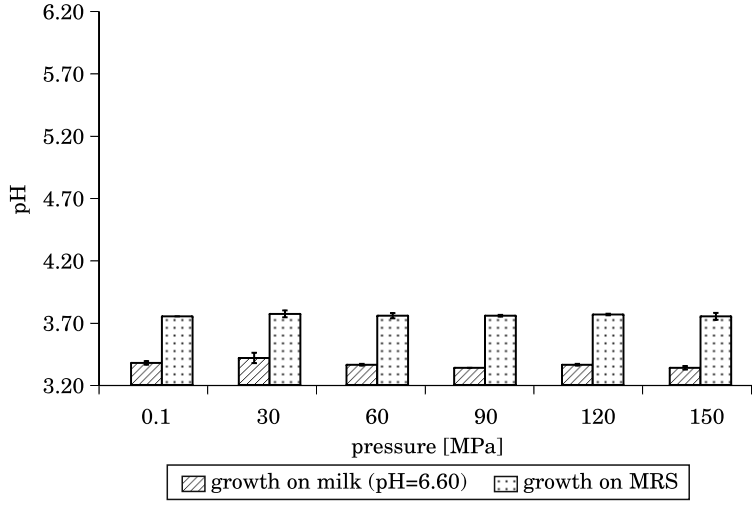

Fig. 5. Acidifying activity of *Lactobacillus acidophilus 132/2* – II passage

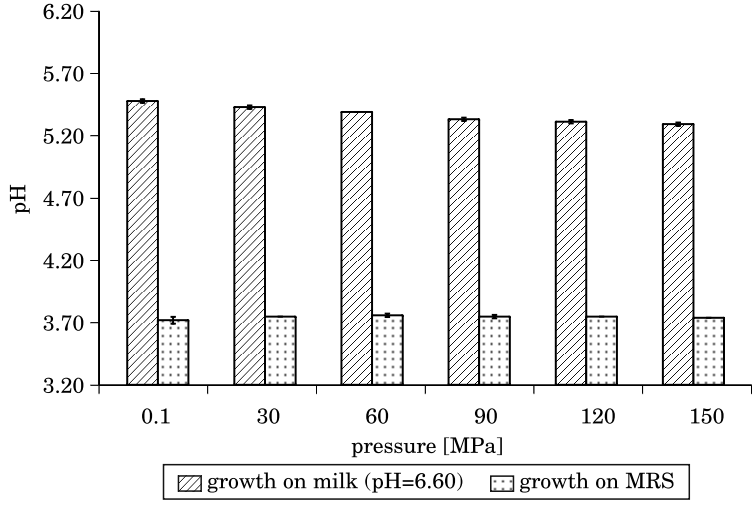

Fig. 6. Acidifying activity of *Lactobacillus acidophilus 294* – II passage

In turn, a study by KRASOWSKA et al. (2005) demonstrated that the higher the value of the pressure applied, the lower the content of lactic acid in the culture of pressurized bacteria. Pressurization at 300 MPa for 15 min did not cause any significant decrease in the acidifying activity. Only the pressure treatment of 500 MPa/1 min was found to considerably inhibit the acidifying activity of the examined bacteria of *Lactobacillus helvetius*.

Effect of pressurization on the antibacterial activity of the examined strains of *Lactobacillus acidophilus*

The antibacterial activity of the investigated strains was higher in cultures run on MRS medium than in those run on milk. (Table 1 and Table 2).

The highest antibacterial activity was reported for the strain *Lactobacillus acidophilus T132/2*. Pressurization was found not to affect its antibacterial activity, either immediately after pressurization in passage I or after inoculation in passage II, in culture on milk. In contrast, the antibacterial activity was observed to increase in the culture run on the MRS medium immediately after pressurization.

Pressurization did not evoke any changes in the antibacterial activity of the strain *Lactobacillus acidophilus LA-5* in cultures on milk. In turn, its increase was observed in the culture on the MRS medium both immediately after pressurization in passage I as well as after inoculation in passage II.

The weakest antibacterial activity was exhibited by the strain *Lactobacillus acidophilus T294*. In the culture on milk in passage I it did not inhibit the growth of three, whereas in passage II it inhibited the growth of two out of the 11 test strains. In the culture on the MRS medium, the *Lactobacillus acidophilus T294* strain inhibited the growth of all test strains. After inoculation in passage II, an increase in the antibacterial activity was observed in the culture run on the MRS medium.

The antibacterial activity is determined, to a significant extent, by the amount of lactic acid produced by bacteria, which is linked to their acidifying activity. A decrease in pH to a value of 4.2–4.5 is generally recognized as sufficient to inhibit the growth of putrefactive bacteria, butyric bacteria and enteropathogens. (WOJTATOWICZ and CHRZANOWSKA, 1998) High-pressure homogenization in a pressure range of 50–150 MPa evokes an increase in the acidifying activity of selected strains of the species *Lactobacillus sp.,* which results in an increase in the antibacterial activity against pathogenic microflora. (LANCIOTTI et al. 2007).

Conclusions

– pressurization in a pressure range of 30–150 MPa/ 1 min did not exert any significant effect on the survivability of the examined strains of *Lactobacillus acidophilus;*

– cultures of the examined strains of *Lactobacillus acidophilus* run on milk exhibited lower resistance to a pressure treatment at 30–150 MPa/ 1 min, as compared to the cultures on liquid MRS medium;

Table 1

Antibacterial activity of the *Lactobacillus acidophilus* T132/2 strain – I passage

Pressure [MPA]	Growth medium	*Escherichia coli 366*	*Escherichia coli 345*	*Escherichia coli L1*	*Enterobacter cloacae 6*	*Enterobacter cloacae 10*	*Enterobacter cloacae 11*	*Enterobacter cloacae 17*	*Proteus 6H*	*Proteus dw*	*Klebsiella ssp. 499*	*Klebsiella pneumoniae B*
		The size of growth inhibition zones of the test strains [mm]										
Passage		I	I	I	I	I	I	I	I	I	I	I
0	milk	12.0 ± 0.0	21.0 ± 1.4	16.5 ± 0.7	17.0 ± 0.0	13.0 ± 0.0	24.5 ± 0.7	13.0 ± 0.0	23.0 ± 0.0	16.5 ± 0.7	16.0 ± 0.0	14.0 ± 0.0
	MRS	22.5 ± 0.7	35.5 ± 0.7	28.5 ± 0.7	29.0 ± 1.4	24.5 ± 0.7	33.5 ± 0.7	30.5 ± 0.7	50.0 ± 0.0	45.0 ± 0.0	44.5 ± 2.1	31.0 ± 0.0
30	milk	13.0 ± 0.0^s	21.0 ± 1.4^n	16.5 ± 0.7^n	17.0 ± 0.0^n	13.5 ± 0.7^n	24.5 ± 0.7^n	13.0 ± 0.0^n	22.5 ± 0.7^n	16.0 ± 0.0^n	15.0 ± 0.0^s	14.5 ± 0.7^n
	MRS	23.0 ± 1.4^n	37.0 ± 1.4^n	28.5 ± 2.1^n	29.5 ± 2.1^n	22.0 ± 0.0^n	33.5 ± 0.7^n	31.0 ± 1.4^n	50.0 ± 0.0^n	44.0 ± 1.4^n	48.0 ± 0.0^s	34.5 ± 0.7^s
60	milk	13.0 ± 0.0^s	20.5 ± 0.7^n	16.5 ± 0.7^n	17.5 ± 0.7^n	14.5 ± 0.7^s	24.5 ± 0.7^n	13.0 ± 0.0^n	23.5 ± 0.7^n	16.5 ± 0.7^n	15.0 ± 0.0^s	15.0 ± 0.0^s
	MRS	26.0 ± 1.4^s	37.5 ± 0.7^n	30.5 ± 2.1^n	32.5 ± 2.1^n	22.5 ± 0.7^n	32.0 ± 0.0^n	31.5 ± 0.7^n	50.0 ± 0.0^n	44.5 ± 0.7^n	49.0 ± 1.4^s	36.0 ± 0.0^s
90	milk	13.5 ± 0.7^s	20.0 ± 0.0^n	17.0 ± 0.0^n	17.0 ± 0.0^n	14.5 ± 0.7^s	26.0 ± 0.0^s	14.0 ± 0.0^n	24.0 ± 1.4^n	15.5 ± 0.7^n	15.5 ± 0.7^n	15.0 ± 0.7^s
	MRS	21.5 ± 0.7^n	38.0 ± 1.4^n	30.0 ± 0.0^n	28.0 ± 0.0^n	24.5 ± 0.7^n	31.5 ± 2.1^n	30.0 ± 0.0^n	50.0 ± 0.0^n	43.0 ± 0.0^s	45.0 ± 0.0^n	35.0 ± 0.0^s
120	milk	13.0 ± 0.0^s	20.5 ± 0.7^n	16.5 ± 0.7^n	16.5 ± 0.7^s	14.0 ± 0.0^n	24.0 ± 0.0^n	13.0 ± 0.0^n	23.5 ± 0.7^n	15.0 ± 0.0^s	15.0 ± 0.0^s	14.0 ± 0.0^n
	MRS	23.0 ± 1.4^n	39.0 ± 1.4^s	30.0 ± 0.0^n	29.5 ± 2.1^n	24.5 ± 2.1^n	31.0 ± 1.4^n	32.0 ± 1.4^n	50.0 ± 0.0^n	42.0 ± 0.0^s	46.0 ± 1.4^n	36.0 ± 1.4^s
150	milk	13.0 ± 0.0^s	21.0 ± 1.4^n	17.5 ± 0.7^n	16.0 ± 0.0^s	14.0 ± 0.0^n	26.5 ± 0.7^s	14.0 ± 0.0^n	22.5 ± 0.7^n	15.5 ± 0.7^n	15.5 ± 0.7^n	14.0 ± 0.0^n
	MRS	24.5 ± 0.7^n	37.5 ± 0.7^n	32.0 ± 0.0^s	31.0 ± 1.4^n	24.0 ± 1.4^n	33.5 ± 0.7^n	30.5 ± 0.7^n	50.0 ± 0.0^n	42.5 ± 0.7^s	50.0 ± 0.0^s	37.5 ± 2.1^s

n – not significant; s – significant, $P \leq 0.05$

Table 2

Antibacterial activity of the *Lactobacillus acidophilus* T132/2 strain – II passage

Pressure [MPA]	Growth medium	*Escherichia coli 366*	*Escherichia coli 345*	*Escherichia coli L1*	*Enterobacter cloacae 6*	*Enterobacter cloacae 10*	*Enterobacter cloacae 11*	*Enterobacter cloacae 17*	*Proteus 6H*	*Proteus dw*	*Klebsiella ssp. 499*	*Klebsiella pneumoniae B*
		The size of growth inhibition zones of the test strains [mm]										
Passage		II	II	II	II	II	II	II	II	II	II	II
0	milk	17.0 ± 0.0	23.0 ± 0.0	22.5 ± 0.7	21.0 ± 1.4	16.0 ± 0.0	25.0 ± 0.0	19.5 ± 0.7	36.5 ± 0.7	32.5 ± 0.7	30.5 ± 2.1	30.0 ± 0.0
	MRS	28.0 ± 0.0	39.0 ± 1.4	34.5 ± 2.1	47.5 ± 0.7	26.0 ± 1.4	32.5 ± 0.7	31.5 ± 2.1	53.5 ± 2.1	54.0 ± 2.8	55.0 ± 1.4	29.5 ± 0.7
30	milk	17.5 ± 0.7^n	23.0 ± 0.0^n	21.0 ± 0.0^n	20.0 ± 0.0^n	17.0 ± 0.0^s	23.0 ± 0.0^s	19.0 ± 0.0^n	35.5 ± 0.7^n	32.0 ± 0.0^n	31.5 ± 0.7^n	30.0 ± 0.0^n
	MRS	29.5 ± 2.1^n	39.0 ± 1.4^n	35.5 ± 2.1^n	48.5 ± 0.7^n	24.5 ± 2.1^n	31.5 ± 0.7^n	31.0 ± 1.4^n	54.5 ± 0.7^n	49.0 ± 1.4^s	52.5 ± 3.5^n	32.0 ± 1.4^s
60	milk	17.0 ± 0.0^n	25.0 ± 0.0^s	21.0 ± 0.0^n	21.0 ± 0.0^n	16.0 ± 0.0^n	24.0 ± 1.4^n	19.0 ± 0.0^n	36.0 ± 0.0^n	32.5 ± 0.7^n	28.5 ± 0.7^n	29.0 ± 1.4^n
	MRS	28.0 ± 0.0^n	37.0 ± 1.4^n	33.0 ± 1.4^n	40.0 ± 0.0^s	23.0 ± 1.4^s	28.0 ± 0.0^s	31.5 ± 2.1^n	54.5 ± 0.7^n	50.0 ± 0.0^n	55.0 ± 1.4^n	28.0 ± 0.0^n
90	milk	19.0 ± 0.0^s	24.0 ± 0.0^s	22.0 ± 0.0^n	20.0 ± 1.4^n	16.0 ± 0.0^n	24.0 ± 0.0^n	19.5 ± 0.7^n	36.5 ± 0.7^n	33.5 ± 0.7^n	30.0 ± 0.0^n	32.0 ± 0.0^s
	MRS	26.5 ± 0.7^n	36.0 ± 0.0^s	31.5 ± 0.7^n	45.5 ± 3.5^n	26.5 ± 0.7^n	28.0 ± 2.8^s	31.0 ± 1.4^n	49.5 ± 2.1^s	49.0 ± 0.0^s	55.0 ± 0.0^n	29.0 ± 0.0^n
120	milk	18.0 ± 0.0^s	23.5 ± 0.7^n	21.0 ± 1.4^n	20.5 ± 0.7^n	17.5 ± 0.7^s	23.5 ± 0.7^n	20.0 ± 0.0^n	37.5 ± 0.7^n	36.5 ± 0.7^s	30.0 ± 0.0^n	30.0 ± 0.0^n
	MRS	25.0 ± 1.4^s	36.0 ± 0.0^s	32.5 ± 0.7^n	40.5 ± 0.7^s	27.0 ± 1.4^n	30.5 ± 0.7^n	27.0 ± 1.4^s	51.0 ± 1.4^n	49.0 ± 1.4^s	50.0 ± 0.0^s	30.0 ± 0.0^n
150	milk	19.0 ± 0.0^s	25.0 ± 0.0^s	21.0 ± 0.0^n	21.5 ± 0.7^n	18.0 ± 0.0^s	24.5 ± 0.7^n	20.0 ± 0.0^n	38.0 ± 0.0^s	37.0 ± 0.0^s	30.0 ± 0.0^n	32.0 ± 0.0^s
	MRS	28.0 ± 0.0^n	37.0 ± 0.0^n	33.0 ± 1.4^n	49.0 ± 1.4^s	26.5 ± 0.7^n	32.0 ± 2.8^n	31.0 ± 1.4^n	49.0 ± 1.4^s	54.0 ± 2.8^n	56.0 ± 0.0^n	32.5 ± 0.7^s

n – not significant; *s* – significant, $P \leq 0.05$

– acidifying activity of *Lactobacillus acidophilus* strains of the culture run on milk was higher than that of the culture established on the liquid MRS medium;

– an increase was observed in the acidifying activity of the strain *Lactobacillus acidophilus T294* after pressurization of the culture run on milk;

– antibacterial activity of *Lactobacillus acidophilus* in the culture established in the MRS medium was higher than that of the culture run on milk;

– pressurization was observed to evoke a slight increase in antibacterial activities of the examined strains of *Lactobacillus acidophilus*.

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