UDK 579.67:579.864

T. A. Ishchenko, T. V. Gordievskaya, I. V. Strashnova, G. V. Yamborko, N. Yu. Vasylieva

Odesa I. I. Mechnikov National University, Biological Faculty, Department of Microbiology, Virology and Biotechnology, Dvoryanskaya st., 2, 65082, Odesa, Ukraine, e-mail: jamborkoann@ukr.net

SEARCH FOR NEW BIOTECHNOLOGICALLY VALUABLE LACTOBACILLI STRAINS

Abstract

Aim. *The aim of this work was to study the antibacterial potential and salt tolerance of lactobacilli strains isolated from different sources.* ***Methods.*** *The antagonistic activity of lactobacilli was determined by the diffusion method in the agar. The stability to NaCl was carried out by determining the optical density of the microorganisms suspension in the MRS liquid medium with an appropriate concentration of sodium chloride.* ***Results.*** *Lactobacilli strains were observed the highest degree of antagonistic activity to E. coli and P. aeruginosa test cultures. C. albicans was the weakest sensitive culture. The most active were strains isolated from self-fermenting eggplants (Odesa region). The vast majority of lactobacilli was tolerant at 2.5-5.0 % NaCl. The increasing NaCl concentration to 7.5 % has resulted in a decrease of the viable cells number and its growth intensity. The primary source of strain isolation does not affect the final resistance to NaCl and antagonistic activity. The resistance to NaCl is not related to the antagonistic activity of most lactobacilli strains.* ***Conclusion.*** *Increased content of sodium chloride in nutrient medium don't inhibit the growth of the investigated lactobacilli strains and, moreover, don't affect on their antagonistic ability, which is very important and essential for the creation and production of probiotic products with functional purpose.*

Key words: lactobacilli, probiotic properties, antagonistic activity, salt tolerance, clustering.

Lactic acid bacteria belonging to the genus *Lactobacillus* today are one of the most important and perspective biotechnology objects. These microorganisms attract the close attention for a long time, and their careful study are due, first of all, to the variety of positive effects provided on the human body and animals [1, 8, 12]. The results of numerous experimental and clinical studies indicate a pronounced prophylactic and therapeutic efficacy of probiotic and functional nutrition products based on industrial lactobacilli strains [6, 9, 11].

Nevertheless, the search for new strains of bacteria of the genus *Lactobacillus* to create modern probiotic and products of functional nutrition remains actual. The use of lactobacilli new strains in biotechnology for the production of probiotics becomes possible only after a detailed study of their biological properties, in particular antagonistic activity [3, 4]. Moreover, a great attention is paid to a number of technological properties, particularly of salt tolerance [12].

The purpose of this work was to study the antibacterial potential and salt tolerance of lactobacilli strains isolated from different sources.

Material and methods

34 lactobacilli strains isolated from self-fermenting vegetables, raw meat material and children feces, and 6 strains from the culture collection of microorganisms of the Department of Microbiology, Virology and Biotechnology Odesa I. I. Mechnikov National University (*Lactobacillu buchneri* ATCC 4005, *L. acidophilus* ATCC 32200, *L. plantarum* VTCC 0921, *L. plantarum* UCM B 11/16 та *L. plantarum* UCM B 2209) were used in experiments.

The antagonistic activity of lactobacilli was determined by the diffusion method in the agar [2]. Eukaryotic and prokaryotic microorganisms were used as the test cultures: *Candida albicans* UCM У 2501^{T}, *Esherchia coli* UCM B 906, *Bacillus subtilis* ONU-24, *Pseudomonas aeruginosa* ONU-211, *Staphylococcus aureus* ONU-223. The evaluation of the results was carried out by measuring the diameter of the lack of growth zone, oriented towards the zone of complete suppression of visible growth.

The degree of antagonistic activity was determined by the following criteria: the diameter of the zone of growth absence 1–15 mm means low antagonistic activity; 16–25 mm – medium antagonistic activity; 25 mm and more – high antagonistic activity.

The stability of lactobacilli to NaCl (at concentrations of 2.5 %, 5.0 %, and 7.5 %) was carried out by determining the optical density of the suspension of microorganisms in a MRS liquid medium with an appropriate concentration of sodium chloride. The measurements were carried out by photometric method at λ- 600 nm using a spectrophotometer (model SmartSpecM Plus Spectrophotometer, series 273 BR 05027).

The study was conducted in triplicates. Statistical analysis of the results were performed using *Excel* and *STATISTICA* 8.

Values are reported as the mean ± standard error of the mean (SEM). The Students' t-criterion was used during the comparative analysis of the research results. The p-value < 0.05 was considered statistically significant [5].

Results and discussion

One of the main criteria for the selection of bacterial strains - candidates for the probiotics – is their antagonistic activity to opportunistic and pathogenic microorganisms [7]. The obtained results indicated that the lactobacilli strains inhibited the growth and reproduction of all indicator microorganisms.

However, the degree of antagonistic activity was different and depended predominantly on a test strain and an indicator strain, but not on a source of lactobacilli isolation. *S. aureus* and *C. albicans* were the most resistant to all studied lactobacilli.

The results of determining the degree of lactobacilli antagonistic activity are shown in Table 1.

The most sensitive test cultures were *E. coli* and *P. aeruginosa.* The percentage of antagonistic activity to *E. coli* was 46.2 %, *P. aeruginosa* – 43.7 %.

B. subtilis and *S. aureus* were less susceptible test cultures. The percentage of lactobacilli antagonistic activity to these microoorganisms was only 10.3 % and 7.7 %, respectively. However, the percentage of the medium degree of antagonistic activity was 38.4 % and 33.3 %.

Table 1

Lactobacilli antagonistic activity to test cultures

Test cultures	Degree of antagonistic activity							
	Low		Medium		High		Absence of antagonism	
	Abs.	%	Abs.	%	Abs.	%	Abs.	%
E. coli	0	–	9	23.1	18	46.2	12	31.0
B. subtilis	2	5.1	15	38.4	4	10.3	18	46.0
S. aureus	3	7.7	13	33.3	3	7.7	20	51.0
P. aeruginosa	5	12.8	6	15.2	17	43.7	11	28.0
C. albicans	0	–	1	2.6	0	–	38	97.0

Lactobacilli isolated from self-fermenting eggplants (Odesa region) accounted for the highest percentage (56.0 %) among all examined strains with high degree of antagonistic activity to at least one of the test cultures (Fig. 1).

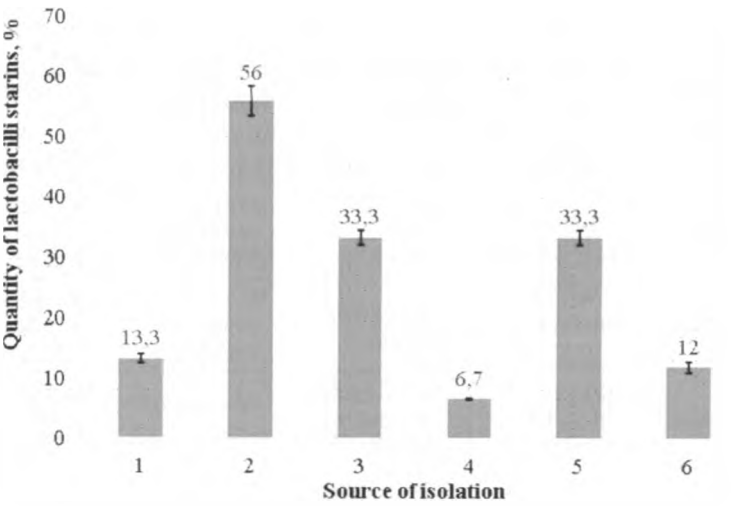

Fig. 1. Lactobacilli strains with high antagonistic activity, isolated from different sources. 1 – children's feces, 2 – self-fermenting eggplants (Odesa region), 3 – raw meat materials (Odesa region), 4 - self-fermenting vegetables (Vietnam), 5 – self- fermenting cucumbers (Sweden), 6 – typical collection strains

Note: high antagonistic activity of lactobacilli strains was expressed in relation to at least one of the test cultures

The lactobacilli strains from raw meat materials (Odesa region) and self-fermenting cucumbers (Sweden) exhibited an equally high degree of antagonistic activity (33.3 %). The percentage of strains from children feces (Odesa region) and typical collection lactobacilli was 13.3 % and 12.0 %, respectively. The smallest percentage (6.7 %) of strains with a high antagonistic activity was selected among lactobacilli isolated from self-fermenting vegetables (Vietnam).

Lactobacilli strains were observed the highest degree of antagonistic activity to *E. coli* and *P. aeruginosa* test cultures.

B. albicans was the weakest sensitive culture. The most active were strains isolated from self-fermenting eggplants (Odesa region).

Probiotic bacteria must adupt well in the gastrointestinal tract. Therefore, the lactobacilli strains ability to survive at different values of NaCl was investigated *in vitro*. The obtained results have confirmed the assumption that lactic acid bacteria undergo stress in an aggressive environment which affects on their viability and survival. Testing for a selective feature – resistance to increased NaCl concentration – is due to the fact that sodium chloride is necessary for the formation of hydrochloric acid, which is an integral part of gastric juice. The vast majority of lactic acid bacteria was tolerant at 2.5–5.0 % NaCl. The increasing NaCl concentration to 7.5 % has resulted in a decrease of the viable cells number and its growth intensity. The validity of the difference between the averaged indices of resistance of the investigated strains to different concentrations of NaCl was performed using a non-parametric analogue of the Student Criterion – a Wilcoxon rank test for two independent samples. It has been shown that the calculated criteria (Table 2) with high probability confirm the difference of the growth rates of strains at concentrations of NaCl 5.0 % and 7.5% compared with control and between each other.

Table 2

Comparison of averaged stability indexes of investigated strains to different NaCl concentrations according to Wilcoxon criteria

Indexes	Control	2.5 % NaCl	5.0 % NaCl	7.5 % NaCl
Control	W = 84.5, p-value = 1	W = 81, p-value = 0.8776	W = 165.5, p-value = 3.623e-05	W = 169, p-value = 1.628e-05
2.5 % NaCl	W = 81, p-value = 0.8776	W = 84.5, p-value = 1	W = 165.5, p-value = 3.634e-05	W = 169, p-value = 1.622e-05
5.0 % NaCl	W = 165.5, p-value = 3.623e-05	W = 165.5, p-value = 3.634e-05	W = 84.5, p-value = 1	W = 163.5, p-value = 5.599e-05
7.5 % NaCl	W = 169, p-value = 1.628e-05	W = 169, p-value = 1.622e-05	W = 163.5, p-value = 5.599e-05	W = 84.5, p-value = 1

The average growth rate of strains in the presence of 2.5 % NaCl also significantly differed from that for experiments with 5.0 % and 7.5 % NaCl. However, for a concentration of NaCl of 2.5 %, the calculated Wilcoxon criterion indicated for the need to adopt a null hypothesis, that is, the absence of a difference between growth indices in control and experiment.

Graphical representation of the averaged indicators of stability of the investigated strains to different concentrations of NaCl has shown at Figure 2.

Just as in the determination of antagonistic activity, it was noted that the primary source of strain isolation does not affect the final resistance to NaCl. For example, among the most resistant strains are present as isolated from self-fermenting vegetables (*Lactobacillus sp.* B4, *Lactobacillus sp.* B1), meat products (*Lactobacillus sp.* M6) and children's feces (*Lactobacillus sp.* 175).

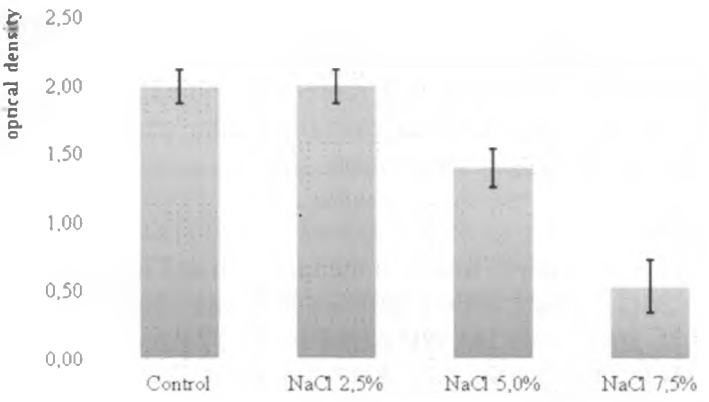

Fig. 2. The averaged indices of investigated lactobacilli resistance to various concentrations of NaCl

To confirm, the clasting with a binary dendrogram construction ("dendextend" package) was performed [10]. This way of visualizing the similarities and differences is to draw two dendrograms like root trees and reflect connecting lines between vertices that correspond to each other in two trees.

The resistance to NaCl is not related to the antagonistic activity of most lactobacilli strains. Only for some strains was placement in parallel (on topology of trees) clusters (*Lactobacillus sp.* M6, *Lactobacillus sp.* M2, *Lactobacillus sp.* M3 and *Lactobacillus sp.* B6, *Lactobacillus sp.* B3) (Fig.3).

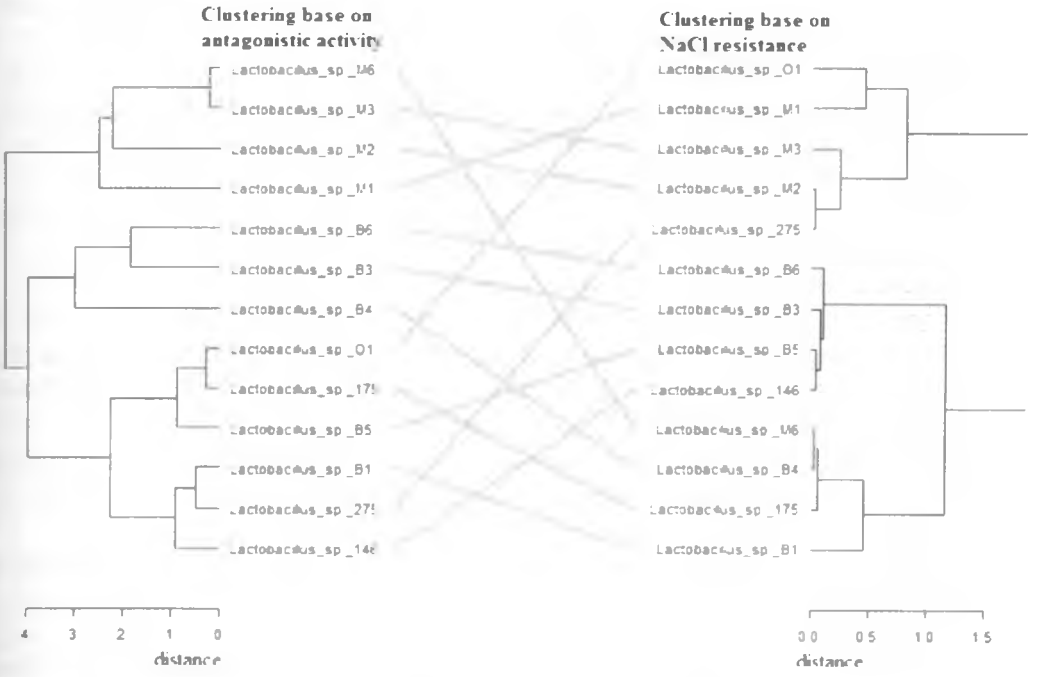

Fig. 3. A binary dendrogram illustrating the trees topology, constructed on the basis of the results of clusterization of the parameters of antagonistic activity and NaCl tolerance (distance matrix – "canberra", mode of aggregation – "complete")

The performed experimental studies and mathematical calculations show that increased content of sodium chloride in nutrient medium don't inhibit the growth of the investigated lactobacilli strains and, moreover, don't affect on their antagonistic ability, which is very important and essential in the creation and production of probiotic products with functional purpose.

References

1. Kitaevskaya S. V. Modern trends in the selection and identification of probiotic strains of lactic acid bacteria // Bulletin of Kazan Technological University. – 2012. – V.15, № 17. – P. 184 – 188 (in Russian).
2. Netrusov A. I., Egorova M. A., Zakharchuk L. M. Workshop on Microbiology: a training manual for students of higher institutions. – Moscow: Academy, 2005. – P. 467 – 478 (in Russian).
3. Ramonova E. V. Isolation and identification of local strains of lactic acid microorganisms and their use as probiotics: Author. diss. cand. biol. sciences. - Vladikavkaz, 2011. – 19 p. (in Russian).
4. Rzhevskaya V. S., Oturina I. P., Teplitskaya L. M. Studying the biological properties of lactic acid bacteria strains // Scientific notes of the V. I. Vernadsky Tauride National University. – 2014. – V. 27, № 1. – P. 145–160 (in Russian).
5. Sidelev S. I. Mathematical methods in biology and ecology: an introduction to elementary biometry: A manual. – Yaroslavl: YarSU, 2012. – 140 p. (in Russian).
6. Solov'eva I. V., Tochilina A. G., Novikova N. A., Belova I. V., et al. Studying the biological properties of new strains of the genus *Lactobacillus* // Bulletin of the N. I. Lobachevsky Nizhny Novgorod University. – 2010. – №. 2 (2). – P. 462 – 468 (in Russian).
7. Tochilina A. G., Belova I. V., Solovyova I. V., Novikova N. A., Ivanova T. P., et al.. Study of the biological properties of strains of the genus *Lactobacillus* // Modern problems of science and education. – 2015. – № 5. (in Russian).
8. Fedorova O. V., Yunusova Z. S., Shurbina M. Yu., Valeeva R. T. Probiotic preparations: characteristics, criteria, requirements for them // Bulletin of the Technological university. – 2016. –Vol. 19, № 7. – P. 142 –145 (in Russian).
9. Conway P. L. Prophylactic treatment of piglets with *Lactobacillus* strains of porcine origin // In: Probiotics: prospects of the use in opportunistic infections. Old Herborn University Seminar Monograpg. Inst. Microbiol. Biochem. Herborn Dill, Germany, 1995 – P. 89–100.
10. Galili T. Dendextend: an R package for visualizing, adjusting and comparing trees of hierarchical clustering// Bioinformatics. – 2015. –Vol. 31(22). – P. 3718–3720.
11. Giraffa G., Chanishvili N., Widyastuti Y. Importance of lactobacilli in food and feed biotechnology // Res. Microbiol. – 2010. – V.161, N 6. – P.480 – 487.
12. Saarela M., Morgensen G., Fondén R., Mättö J., Mat-tila-Sandholm T. Probiotic bacteria: safety, functional and technological properties // Journal of Biotechnology. – 2000. –Vol. 84. – P. 197–215.