Selection of Promising Lactobacilli Antagonistic to Campylobacter Jejuni

Svetlana Makavchik, Larisa Karpenko, Yuri Kuznetsov, Ilya Nikonov, Alesya Bakhta

Abstract**:** ***This article reflects the results of a study on the selection of promising lactobacilli antagonistic to Campilobacter jejuni, a strain that is the most common and more pathogenic for humans, carried out as part of a project to scientifically substantiate the use of new technologies in poultry feeding using special probiotic strains that increase productivity and obtaining poultry products of improved quality with the properties of functional food products. During the study, strains of lactic acid bacteria were obtained. The cultivation of strains of Lactobacillus crispatus, Lactobacillus gasseri, Lactobacillus fermentum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus plantarum was carried out on liquid and agarized nutrient media MRS at 370C for 24 hours. In vitro antagonistic studies were performed using the two-way antagonistic method on a wide range of indicator crops. Since there is evidence of a specific mechanism for the manifestation of the antagonistic activity of lactobacilli to gram-negative and gram-positive bacteria, we used test strains of both groups of bacteria. The antagonistic activity of the studied cultures against pathogenic and opportunistic bacteria was determined by the zone of growth inhibition of indicator strains around the colonies of individual strains of lactobacilli and their consortium (in mm). Priority clinical isolates isolated from birds with intestinal infections were used as indicator cultures: Proteus vulgaris, Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, Campylobacter jejuni and Campylobacter jejuni ATCC strains 33560. During the study, most bacterial strains of the genus Lactobacillus were highly antagonistic its activity against indicator strains. The most sensitive to the inhibitory effect of lactobacilli were E. coli, Campylobacter jejuni, S. typhimurium and P. vulgaris. The research results showed that the strain L.plantarum ATCC 8014 exhibits a more pronounced antagonistic activity than other strains of lactobacilli.***

Keywords **:** ***lactobacilli, antibiotic resistance, antibiotics, broilers, antagonism to Campylobacter jejuni..***

I. INTRODUCTION

The fight against zoonoses transmitted from animals and birds to humans is a serious problem throughout the world. Particular attention in the USA and EU countries is given to campylobacteriosis. One of the routes of transmission is the supply of poultry products, since today there is still a high probability of infection of chickens during their cultivation, and chicken eggs are often subjected to endogenous and exogenous infection, with a change in the composition of the shell and a decrease in the protective components of egg protein, as well as it should be noted that chickens raised in industrial poultry have no colonization resistance of the intestine against Campylobacter spp. One way to solve this problem is using new technologies for poultry nutrition using special probiotic strains is ensured, which provides increased productivity and improved quality poultry products with the properties of functional nutrition products. This study is devoted to one of the stages of developing the scientific foundations of this technology, and in particular, the selection of promising lactobacilli antagonistic to Campilobacter jejuni, a strain that is the most common and more pathogenic for humans. The mechanism of colonization of the intestines of birds and humans by C. jejuni cells has not been studied much. Hendrixson DR and DiRita VJ, 2001, identified in C. jejuni two main genes docB (Cj0019c) and docA (Cj0020c), which provide colonization of the intestine. Numerous chemoattractants present in the intestines enhance the colonization of C. jejuni. The main attractant is mucin [2]. C. jejuni has two main genes, capA and cadF, responsible for the adhesion of a bacterial cell to the intestinal epithelium of birds and humans [3]. In the process of adhesion of C. jejuni on the surface of bird intestinal cells, fibronectin-like protein A, encoded by the flpA gene, is also involved [5]. The biological properties of C. jejuni, such as the colonization and signal transduction necessary for bacterial growth and disease development, can be disrupted by probiotics [6]. Therefore, at present, research by scientists is focused on the use of probiotics for the prevention and treatment of gastrointestinal tract infections. In experiments on one-day chickens [7], as well as on immunodeficient and immunocompetent mice [9], it was shown that the cocktail of probiotic strains inhibited the colonization and growth of C. jejuni. The microaerophilic environment of the intestine promotes the growth and reproduction of C. jejuni. The presence of probiotics inhibits the growth and reproduction of C. jejuni, and also inhibits the penetration of the pathogen into the submucosal layer [10, 11]. It was found that exposure of C. jejuni cells with a probiotic disrupted the pathogen mobility and its ability to colonize intestinal epithelium in vitro and in vivo experiments [11].

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It was found that bacteriocins produced by probiotic strains of L. salivarius and Paenibacillus polymyxa act as colonizing peptides, facilitating the introduction and / or dominance of the producer in the niche already occupied by him [12]. These probiotic strains showed pronounced antagonistic activity against C. jejuni in vitro, but were inactive in vivo. Along with mobility and adhesion, invasion is an important step in the pathogenesis of campylobacteriosis. Probiotics are able to reduce invasion due to their competitive properties, which was reflected in the works of Parkhill J, et al., 2000, Ziprin RL et al., 2001, Hofreuter D, et al., 2006, and studies in which coaggregation of lactobacilli with C. jejuni [14].

Further studies will determine the most promising probiotic strains effective in inhibiting the growth and stimulation of elimination of C. jejuni in industrial poultry, which is an enteropathogen for humans, to improve the quality and safety of poultry products.

II. MATERIALS AND METHODS

The aim of this study was to select promising lactobacilli antagonistic to Campilobacter jejuni. To solve this problem, strains of lactic acid bacteria were obtained. The strains of Lactobacillus crispatus, Lactobacillus gasseri, Lactobacillus fermentum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus plantarum were grown on liquid and agarized MRS culture media at 37 ° C for 24 hours. The cultivation of lactic acid bacteria was carried out at 37 ° C under microaerophilic conditions using a desiccator and a gas generating batch system. The study of antagonistic activity in vitro was carried out by the method of two-layer agar, by the method of blocks, by the method of wells on a wide range of indicator cultures. Since there is evidence of a specific mechanism for the manifestation of the antagonistic activity of lactobacilli to gram-negative and gram-positive bacteria, we used test strains of both groups of bacteria. The antagonistic activity of the studied cultures against pathogenic and opportunistic bacteria was determined by the zone of growth inhibition of indicator strains around the colonies of individual strains of lactobacilli and their consortium (in mm). Priority clinical isolates isolated from birds with intestinal infections were used as indicator cultures: Proteus vulgaris, Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, Campylobacter jejuni and Campylobacter jejuni ATCC strains 33560.

III. RESULT AND DISCUSSION

The strains of L. plantarum ATCC 8014, L. fermentum ATCC 9338, L reuteri ATCC 23272 and their consortium are characterized by the following features “Fig. 1”:

Fig. 1. Example of growing Lactobacillus plantarum on the surface of an MRC agar medium.

1. Cultural traits: when grown on the surface of an agar medium MRC pH-6.5 (Himedia, India) in an incubator at 37 ° C for 24-48 hours under microaerophilic conditions: Lactobacillus crispatus strain - medium, pale white, round colonies , flat with an even edge, Lactobacillus gasseri strain - small, pale white, round colonies, flat with an even edge, L. plantarum ATCC 8014 colony - medium, pale white, round colonies, flat with an even edge, L. fermentum ATCC strain 9338 - colonies are small, pale white, round, flat with a smooth edge, strain L reuteri ATCC 23272 - colonies are medium, white, round, ypuklye with smooth edge.

Morphological signs: when grown on liquid MPC pH-6.5 (Himedia, India) or milk at 37 ° C for 24-48 hours, cells of the L. fermentum strain are medium bacilli ~ 5-6 microns in size, form short chains ; cells of strain L reuteri are medium rods of size ~ 4-5 microns, form chains, cells of strain L. plantarum are short rods of size ~ 3 microns, form short chains.

Physiological and biological characteristics: all strains are facultative anaerobes, temperature optimum 37 ± 2 ° C, grow at 42 ° C, weak growth at 30 ° C. The optimum pH value is 5.5-6.0. Priority clinical isolates isolated from birds with intestinal infections were used as indicator cultures: Proteus vulgaris, Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, Campylobacter jejuni and Campylobacter jejuni ATCC strains 33560 “Fig. 3, 4, 5”.

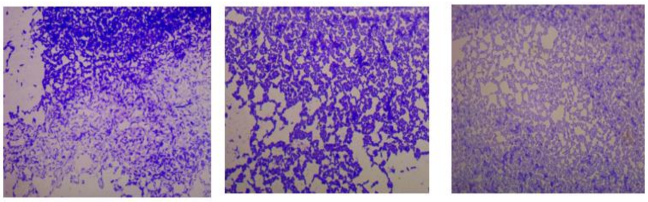

Fig. 2. The results of microscopy of smears on the example of L reuteri (1), L. Plantarum (2). L. reuteri (3).

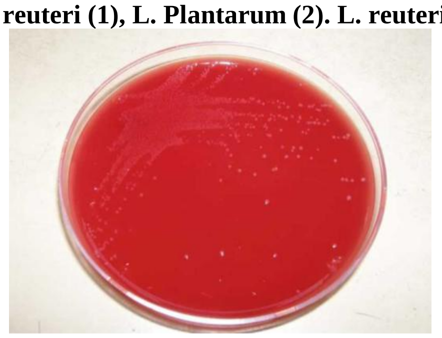

Fig. 3. Growth of Staphylococcus aureus on Meat-Peptone Agar (MPA)

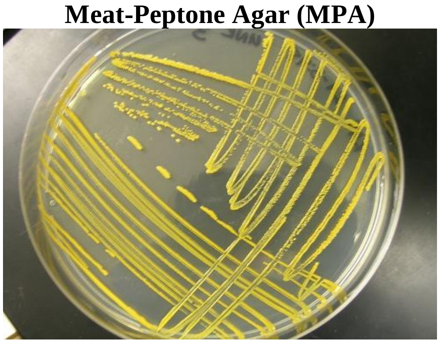

Fig. 4. Growth of Campylobacter jejuni after 72 h of cultivation

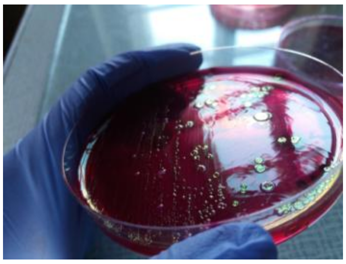

Fig. 5. Growth of Escherichia coli on Endo medium

The antagonistic activity of the cultures in Laclobacillus plantarum, Lactobacillus reuteri and Lactobacillus fermentum and their consortium was studied for clinical isolates: Escherichia coli, Proteus vulgaris, Staphylococcus aureus, Candida albicans, Salmonella typhimurium, Campyunibact. Antagonistic activity was judged by the zone of lack of growth of test strains around the test strain of lactobacilli. One of the main probiotic indicators is antagonism. The most sensitive to the inhibitory effect of lactobacilli were E. coli, Campylobacter jejuni, S. typhimurium and P. vulgaris. The results of the studies indicate that the inventive consortium of strains exhibits more pronounced antagonistic activity than individual strains of Laclobacillus plantarum and Lactobacactobus reuteri fermentum. Analysis of table 1 indicates that most strains of bacteria of the genus Lactobacillus have a high antagonistic activity against indicator strains.

Table- I: Antagonistic activity of individual strains of lactic acid bacteria

Indicator culture	Zones of growth inhibition of indicator microorganisms for individual strains of lactobacilli and their consortium, mm (M ± m)					
	Lactobacillus fermentum ATCC 9338	*Lactobacillus reuteri* ATCC 23272	*Lactobacillus crispatus* BKM B-2727D	*Lactobacillus plantarum* ATCC 8014	*Lactobacillus plantarum* MD IIE-2165	*Lactobacillus gasseri* BKM B-2728D
*Escherichia coli**	4,4±0,6	3,5±0,3	3,9±0,2	5,4±0,9	1,7±0,2	2,6±0,3
Proteus vulgaris *	3,8±0,4	3,6±0,3	3,9±0,5	6,2±0,8	0,9±0,1	1,5±0,1
Campylobacter jejuni ATCC 33560	7,4±1,8	7,0±0,9	2,9±1,2	8,4±1,8	2,9±1,2	1,4±0,2
*Campylobacter jejuni**	6,5±0,4	6,0±0,9	5,4±0,8	8,2±1,8	1,4±0,2	2,4±0,5
Salmonella typhimurium *	6,5±0,4	7,3±0,5	5,4±0,8	8,9±1,8	1,8±0,3	2,9±0,3
Staphylococcus aureus *	4,7±0,3	3,8±0,3	2,9±0,4	6,6±0,7	2,6±0,4	1,6±0,2
*- clinical isolates isolated from birds in bacterial intestinal infections.						

Table- II: Antagonistic activity of cultures of lactic acid bacteria against pathogenic and opportunistic microorganisms

Test - strains	Inactive strains	The number of active strains / degree of inhibition		
		high	average	low
E. coli	5	73*	3**	1
S. typhimurium	9	68*	3	4
P. vulgaris	12	62*	5	1
S. aureus	7	64	10	3
C. jejuni	7	70*	6**	1
* - p < 0,001; ** - p < 0,05				

Analysis of table 2 allows us to conclude that the studied cultures are able to inhibit pathogenic microflora. So, 88% of lactobacilli inhibit E. coli (p <0.002), 83% inhibit the growth of Campylobacter jejuni (p <0.001): thus, the bulk of lactobacilli cultures studied in this study have antagonistic activity against a number of pathogenic and conditionally pathogenic microorganisms. The results of the study showed that the active acidity of the medium affects the antagonistic activity of the studied strains of lactobacilli in relation to strains of the test culture of E. coli. It was noted that the stronger the pH of the medium is shifted to the acidic side, the stronger the antagonistic effect, and the stronger the pH of the medium is shifted to the alkaline side, the weaker the antagonistic effect of the strains studied by us. The greatest antagonistic effect with respect to clinical isolates was observed at pH = 5-6 in almost all strains of lactobacilli. Thus, the active acidity of the medium has a strong effect on the antagonistic activity of acidophilus bacillus strains against Escherichia coli strains. Clinical isolates of E. coli, which are the most resistant to changes in pH of the medium and retain antagonism with respect to the strains.

IV. CONCLUSION

Thus, most bacterial strains of the genus Lactobacillus have high antagonistic activity against indicator strains. The most sensitive to the inhibitory effect of lactobacilli were E. coli, Campylobacter jejuni, S. typhimurium and P. vulgaris. The research results showed that the strain L.plantarum ATCC 8014 exhibits a more pronounced antagonistic activity than other strains of lactobacilli.

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REFERENCES

1. D. R. Hendrixson, B. J. Akerley, V. J. DiRita, Transposon mutagenesis of Campylobacter jejuni identifies a bipartite energy taxis system required for motility. *Mol Microbiol*, 2001, Volume 40, pp.214–224.
2. M. B. Hugdahl, J. T. Beery, M. P. Doyle, Chemotactic behavior of Campylobacter jejuni. *Infect Immun*, 1988, Volume 56, pp. 1560–1566.
3. S. S. Ashgar, N. J. Oldfield, K. G.Wooldridge, M. A. Jones, G. J. Irving, D. P. Turner, D. A. Ala'Aldeen, CapA, an autotransporter protein of Campylobacter jejuni, mediates association with human epithelial cells and colonization of the chicken gut. *J Bacteriol*, 2007, Volume 189, pp. 1856–1865.
4. R. C. Flanagan, J. M. Neal-McKinney, A. S. Dhillon, W. G. Miller, M. E. Konkel, Examination of Campylobacter jejuni putative adhesins leads to the identification of a new protein, designated FlpA, required for chicken colonization. *Infect Immun*, 2009, Volume 77, pp. 2399–2407.
5. M. E. Konkel, J. E. Christensen, A. S. Dhillon, A. B. Lane, R. Hare-Sanford, D. M. Schaberg, C. L. Larson, Campylobacter jejuni strains compete for colonization in broiler chicks. *Appl Environ Microbiol*, 2007, Volume 73, pp. 2297–2305.
6. V. Mohan, The role of probiotics in the inhibition of Campylobacter jejuni colonization and virulence attenuation. *Eur J Clin Microbiol Infect Dis*. 2015. Volume 34, pp. 1503-1513.
7. T. Y. Morishita, P. P. Aye, B. S. Harr, C. W. Cobb, J. R. Clifford, Evaluation of an avian-specific probiotic to reduce the colonization and shedding of Campylobacter jejuni in broilers. *Avian Dis*, 1997, Volume 41, pp. 850–855.
8. W. L. Willis, L. Reid, Investigating the effects of dietary probiotic feeding regimens on broiler chicken production and Campylobacter jejuni presence. *Poult Sci*, 2008, Volume 87, pp. 606–611.
9. R. D. Wagner, S. J. Johnson, D. Kurniasih Rubin, Probiotic bacteria are antagonistic to Salmonella enterica and Campylobacter jejuni and influence host lymphocyte responses in human microbiotaassociated immunodeficient and immunocompetent mice. *Mol Nutr Food Res*, 2009, Volume 53, pp. 377–388.
10. V. F. Aguiar, A. M. Donoghue, K. Arsi, I. Reyes-Herrera, J. H. Metcalf, de los F. S. Santos, P. J. Blore, D. J. Donoghue. Targeting motility properties of bacteria in the development of probiotic cultures against Campylobacter jejuni in broiler chickens. *Foodborne Pathog Dis*, 2013, Volume 10, Issue 5, pp. 435–441.
11. A. Alemka, M. Clyne, F. Shanahan, T. Tompkins, N. Corcionivoschi, B. Bourke, Probiotic colonization of the adherent mucus layer of HT29MTXE12 cells attenuates campylobacter jejuni virulence properties. *Infect Immun*, 2010, Volume 78, Issue 6, pp. 2812–2822.
12. N. J. Stern, B. V. Eruslanov, V. D. Pokhilenko, et al, Role of Campylobacter jejuni potential virulence genes in cecal colonization. *Avian Dis*, 2001, Volume 45, pp. 549–557.
13. D. Hofreuter, J. Tsai, R.O. Watson, et al, Unique features of a highly pathogenic Campylobacter jejuni strain. *Infect Immunol*, 2006, Volume 74, pp. 4694–4707.
14. M. Romero, Coaggregation of Lactobacilli with Campylobacter jejuni. *I-ACES 1*, 2014, Issue 1.

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