Biological Properties of Potential Pathorgenicity in Some Enterobacteria Isolated from Dairy Products

L.G. Molochaeva[1,*], *R.Kh.* Gairabekova[1] and *E.M.* Pshukova[2]

[1]Kadyrov Chechen State University, Grozny, Russia
[2]Kabardino-Balkarian State University Named After Kh.M. Berbekov, Nalchik, Russia

Abstract. Opportunistic microorganisms, which often become the etiological agents of hospital infections, are currently widespread. This problem requires constant attention from specialists to the study of the ways of circulation of these bacteria in the environment, especially those species that have a fecal-oral mechanism of distribution.Studies aimed at detecting bacteria belonging to the Enterobacteriaceae family in milk and dairy products produced in private backyards made it possible to isolate 252 cultures belonging to this family. Among the isolated enterobacteria were representatives of the species Escherichia coli, Enterobacter aerogenes, Enterobacter cloacae, Citrobacter freundii, Serratia marcescens, Yersinia enterocolitica. Among these microorganisms there is a wide variety of strains that differ in ecological properties, range of hosts, pathogenicity for plants, animals and humans. A number of species cause gastrointestinal diseases, i.e. are enteropathogenic.

1 Introduction

The classification of pathogenicity factors according to their purpose and mechanism of action includes pathogenetically significant products of a bacterial cell, which determine the sequence of stages in the development of an infectious process and its outcome. These factors are grouped into four groups: colonization, invasion, toxigenicity, and persistence.

Colonization is the settlement of microorganisms in a particular host biotope. This stage of infection of the body begins with adhesion i.e. attachment of the pathogen to the cells of the body at the entrance gate of infection. The leading role in the attachment of bacteria to sensitive cells of the macroorganism belongs to the adhesive ability of the microorganism.

Hemolysins are substances capable of releasing hemoglobin from red blood cells. In this case, hemoglobin dissolves in the plasma or the surrounding fluid and the blood (or a suspension of erythrocytes) becomes transparent (lacquer blood). Hemolysins are the waste products of many bacteria (staphylococci, streptococci, etc.), parasitic worms, insects, scorpions, and some poisonous snakes (lysolecithins).

The ability to resist non-specific immunity, i.e. to survive in the host organism is associated with the ability of bacteria to produce lysozyme and anti-lysozyme. Lysozyme is

* Corresponding author: l_molochaeva@mail.ru

used to suppress the normal flora of the body, and antilysozyme is used to resist the lysozyme of the host organism. The aim of the work was to identify the ability of the isolated cultures of bacteria to produce lmisozyme, antilysozyme, hemolysis and the ability to hemagglutination.

2 Research methodology

Among the enterobacteria isolated from dairy products were Escherichia coli - 51 strains, Enterobacter aerogenes - 26 strains, Enterobacter cloacae - 21 strains, Citrobacter freundii - 59 strains, Serratia marcescens - 19 strains, Yersinia enterocolitica - 76 strains. [8, 9]

The crops were cultivated at a temperature of 370C in a thermostat. The results were recorded after 24-48 hours for aerobic bacteria and after 48-96 hours for anaerobic bacteria. The adhesive activity of bacteria was determined in the hemagglutination reaction with a 3% suspension of ram erythrocytes in the presence of D-mannose and without it. A positive hemagglutination reaction in the presence of D-mannose was considered as the presence of D-mannose-resistant adhesive activity in bacteria. [1-3, 6, 7, 10, 12, 16-18, 20-23, 25, 27, 28]

Hemolytic activity of bacteria was detected on nutrient agar with the addition of 3-5% washed rabbit erythrocytes. Hemolysis was taken into account after 24-48 hours of incubation. [1-3, 5-7, 10-12, 15, 18-28]

The ability of the studied Escherichia cultures to produce hemolysins produced in the presence of thiols (alcohols containing an SH–group with the general formula R–SH), i.e. hemolysins, the secretion of which is stimulated by thiols in accordance with the recommendations of Albesa et al., were detected on tryptinase-soy agar, to which rabbit erythrocytes washed three times in Hank's solution were added [5, 26]. The results of thiol-dependent hemolytic activity in these bacterial cultures were taken into account after 24-48 hours of incubation in a thermostat.

The ability to produce lysozyme was determined using the method of “delayed antagonism” after growing bacteria on nutrient agar for 24 hours and 10 minutes of treatment with chloroform vapors, layered with a two-layer washing method of a daily agar culture of Micrococcus luteus var. lisideikticus. The results were taken into account after 18-24 hours. [1, 2, 4, 6, 7, 10, 12, 18, 20-23, 25, 27, 28]

The study of antilysozyme activity was carried out according to the method of O.V. Bukharin (1984) [3, 4, 13, 14]. To determine the anti-lysozyme activity in physiological saline, a series of dilutions of lysozyme from egg white (Olainsky Plant of Chemical Reagents) was prepared at concentrations (2, 3, 4, 5, 10, 20, 30, 40, 50 μg/ml). 0.5 ml of each dilution of lysozyme was mixed with 4.5 mg of molten and cooled to 45°C 1.5% nutrient agar and poured into Petri dishes. On agar with lysozyme, drops of daily cultures of the studied strains were applied with a standard loop. The crops were incubated for 18-24 hours at 37°C, then the studied cultures grown in macrocolonies were killed with chloroform vapor for 10 minutes. After that, the macrocolonies were covered with a second layer of 3 ml of 0.7% agar nutrient medium mixed with 0.1 ml of a bacterial suspension of a daily agar indicator culture of Micrococcus luteus (ATCC No 29470, GNIISK named after L.A. Tarasevich), with a turbidity of 4 according to McFarland, and placed in a thermostat at 37°C for a day. The test was considered positive when the indicator strain of Micrococcus luteus grew around the colonies of those strains that neutralized the egg lysozyme added to the agar layer.

3 Results and discussions

In the study of the agglutinating ability of sheep erythrocytes in the absence of D-mannose, it was found that this trait occurs among all species of enterobacteria studied. However, this symptom occurs with different frequency in different types of bacteria.

The smallest number of strains 42 out of 124 (33.87%) with the ability to hemagglutination were identified among cultures of Enterobacter cloacae. The studied cultures of citrobacteria produced hemagglutinins in the absence of D-mannose more often - 41 strains out of 59 studied (69.5%) than in the presence of D-mannose - 4 strains i.e. 6.8%. The ability to agglutinate sheep erythrocytes from 76 tested Yersinia strains was found in 39 cultures (51.3%). The hemagglutinins identified in the studied strains of Yersinia belonged to different groups according to sensitivity to mannose. Thus, agglutination of erythrocytes was suppressed by mannose in 24 cultures (31.6%), and mannose-resistant hemagglutinins were characteristic of 15 strains of the studied bacteria, i.e. 19.7%. In the study of the agglutinating ability of ram erythrocytes in the absence of D-mannose, it was found that this trait is very common in Escherichia cultures - 92.2% of strains from the studied 51 cultures belonging to this species had such properties. The hemagglutinins identified in the studied Escherichia strains belonged to different groups according to sensitivity to mannose. Mannose-resistant hemagglutinins in relation to ram erythrocytes were detected in 13 Escherichia strains (25.5%), agglutinins sensitive to the presence of D-mannose were detected in 34 strains (66.7%).

Among the 19 strains of serrations subjected to the study, 14 cultures were identified that have a hemagglutinating ability in the absence of D-mannose, which is 73.7%. D-mannose-resistant hemagglutination activity was noted in 8 strains of the studied serrations (42.1%).

Identification of the ability of the studied cultures of citrobacter to produce hemolysins showed the presence of this ability in 34 strains, which is 57.6%. Thiol-dependent hemolytic activity was characteristic of 7 strains out of 59 tested cultures i.e. 11.9%.

The conducted studies made it possible to identify among 76 Yersinia strains the ability to lyse rabbit erythrocytes in 48 cultures, which amounted to 63.2%. Thiol-dependent hemolytic activity was detected in 5 strains out of 76 tested cultures (6.6%).

Of the 26 tested strains of Enterobacter aerogenes, 16 cultures were characterized by hemolytic activity, which amounted to 61.5%. Among the 21 tested cultures of Enterobacter cloacae, 9 strains had hemolytic activity, which is 42.9%. Cultures with thiol-dependent hemolytic activity among the studied strains of Enterobacter could not be identified. Studies aimed at identifying hemolytic activity in 51 Escherichia cultures showed that such activity is typical for 49 of them, which is 96.1%. But there were significantly fewer strains with thiol-dependent hemolytic activity among Escherichia, there were only 3 cultures, which corresponds to 5.9%.

4 Conclusions

The ability to lyse rabbit erythrocytes was characteristic of 11 serration cultures out of 19 tested, which is 57.9%. The ability to hemolysis in the presence of L-cysteine (thiol-dependent hemolytic activity) was absent in the tested cultures.

Of 59 cultures of citrobacteria isolated from dairy products, secreting lysozyme was 13 (22.03%).

The ability to produce antilysozyme was characteristic of 27 (45.76%) of the 59 cultures of citrobacteria studied.

Of the 76 cultures of Yersinia enterocolitica studied by us, 12 cultures (15.8%) produced lysozyme. Antilysozyme activity was 38 cultures (65.8%). A quantitative study of anti-lysozyme activity showed that the studied Yersinia cultures are characterized by anti-lysozyme activity in the range from 1 to 6 μg/ml.

We assigned 47 cultures of Enterobacter aerogenes, Enterobacter cloacae species isolated from fermented milk products, 21 cultures belonged to the Enterobacter cloacae species, and 26 strains to the Enterobacter aerogenes species. The ability to produce lysozyme was characteristic of 4 strains of Enterobacter cloacae, which is 19.04%. Among the cultures of Enterobacter aerogenes, there were 7 such strains, which is 26.9%.

51 Escherichia cultures isolated from lactic acid products, of which 19 produced lysozyme - 37.2%, anti-lysozyme activity was characteristic of 47 strains - 92.1%.

No lysozyme-producing strains were found among the 19 studied cultures of serrations isolated from fermented milk products.

The ability to produce antilysozyme was characteristic of 15 strains, out of 19 cultures of serrations isolated from fermented milk products - 78.8%.

References

1. S. K. Aishanov, *Some features of opportunistic bacteria isolated from rabbits suffering from hind limb ulcers*, 260-262 (2011).
2. L. Z. Blieva, Comparative study of bacteria of the genus, **1**, 46 (1996).
3. V. B. Boziev, Correlation between antilysozyme activity and other factors of enterobacteria pathogenicity, 1, 27-28 (1996).
4. I. M. Gabrilovich, *Lysozyme and antilysozyme activities in opportunistic enterobacteria*, 163-164 (1989).
5. I. M. Gabrilovich, *Thiol-dependent hemolysins as a factor in the pathogenicity of bacteria of the genus Citrobacter and Serration*, 69-71 (1990).
6. I. M. Gabrilovich, *Experimental study of toxicoseptic syndrome caused by opportunistic enterobacteria*, 63-64 (1997).
7. R. Kh. Gairabekova, Pathogenic and opportunistic bacteria in milk and dairy products produced at home, 1, 19-23 (2019).
8. R. Kh. Gairabekova, Pathogenic streptococci, staphylococci and opportunistic enterobacteria in milk and dairy products produced at home, **15(1)**, 52-58 (2019).
9. F. S. Turlova, Production of hemolysins by bacteria of the genus Serratia isolated from various sources in the territory of the Czech Republic, **1(17)**, 126-128 (2015).
10. O. S. Yakushenko, *Pathogenicity factors of some opportunistic enterobacteria*, 55-59 (1991).
11. I. Albesa, L. I. Barberis, M. K. Pajaro, M. K. Faraochi, A. J Fraso, A tiol aktivited-hemolisin in gram-negative bacteria, **31**, 297-300 (1985).
12. I. M. Gabrilovich, *Epidemiological markers of opportunistic enterobacteria*, 268 (1990).
13. R. Gayrabekov, Some features of the bacteria of the family enterobacteriaceae allocated from small cattle at different pathological processes, **4(14)**, 7322-7327 (2020).