Lysozyme, anti-lysozyme, hemolytic and adhesive activity in serration cultures isolated from dairy products

R. Kh. Gairabekov[1*], *L. G.* Molochaeva[1], and *N. M.* Mirzoeva[2]

[1] Chechen State UniversityNo. 31, L. Yashin Str., Grozny, 366300, Russian Federation
[2] Kabardino-Balkarian University named after H. M. Berbekov, 73, Chernyshevsky Str., Nalchik, 360004, Russian Federation

Abstract. Isolation of pathogenic or opportunistic bacteria from surrounding environment, food products, animals and humans requires establishing their potential pathogenic potential. In this work, the authors have studied 109 cultures of bacteria belonging to the species Serratia marcescens of which: 61 museum cultures, 29 cultures isolated from small ruminants and environmental objects, 19 cultures isolated from dairy products produced on private farmsteads in the Chechen Republic for the presence of lysozyme, antilysozyme, hemolytic and adhesive activity in them.

1 Introduction

Provision of population of the country with healthy food is an important task afflicting state institutions responsible for national health. An especially important problem is prevention of development of pathogenic and opportunistic microorganisms in food products. It includes prevention of possible contamination of products, prevention of circulation of pathogenic and opportunistic microorganisms in population of a given territory and in the natural ecosystem as a whole, as population is an element of this ecosystem. This problem has become especially relevant to population now, as the population suffers from a pandemic of SARS-COV-2 virus (coronavirus), the worst pandemic for the last 100 years, resulting in death of over a million of people and deep decline in the global economy.

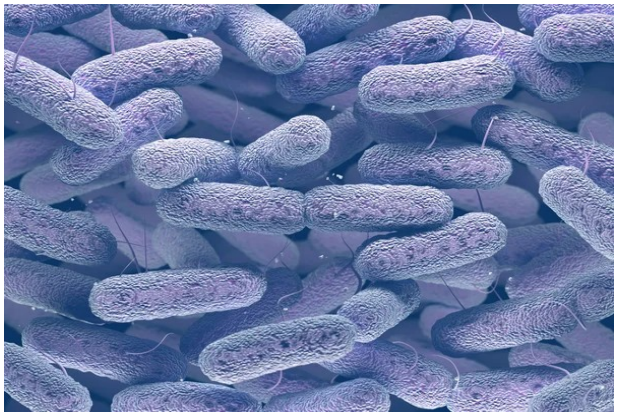

Fig. 1. *Enterobacteria*: a large family of *Gram-negative* bacteria that includes many of the more familiar pathogens, such as *Salmonella pestis, Escherichia Coli, Yersinia, Klebsiella and Shigella, Proteus, Enterobacter, Serratia,* and *Citrobacter*.

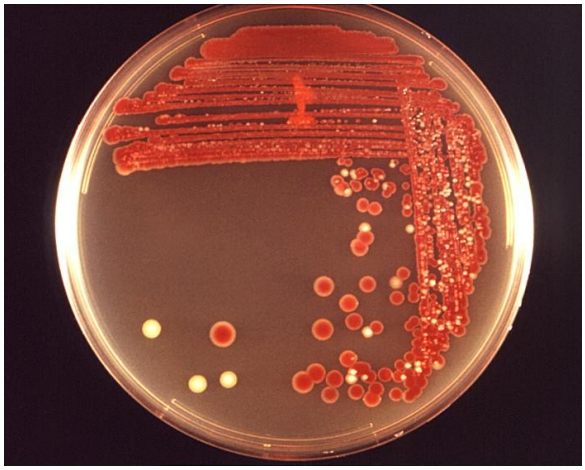

Fig. 2. *Serratia marcescens* under the microscope.

29 bacterial cultures identified as *Serratia marcescens* [9–13] were successfully isolated from sick animals (goats and sheep) and environmental object in the territory of the Chechen Republic.

The authors conducted studies aimed at identification of pathogenic and opportunistic microorganisms in dairy products produced for sale in private farms of the Republic.

The study of dairy products was finalized by isolation of 19 bacterial cultures of *Enterobacteriaceae* (Figure 1) bacterial family, which, after morphological, culture-based and biochemical assay, were identified as bacteria of genus *Serratia*, species *Serratia marcescens* (Figure 2) [7, 8].

2 Materials and methods

This research considered 19 cultures identified as *Serratia marcescens*, isolated from dairy products form private farms, 61 museum cultures and 29 cultures

* Corresponding author: ruslan.sarmat@mail.ru

isolated from goats and sheep and environmental objects in the territory of the Chechen Republic [7–11].

Lysozyme activity was determined by the method of delayed antagonism according to Bukharin et al [2, 4, 9, 13] on 1.5% nutrient agar. Plate cultures were incubated at 37^0C for 18-24 hours, then grown colonies were exposed to chloroform vapors, after that they were subjected to being couched together using two-layer method with washout of one-day culture of *Micrococcus luteus var. Lysodeikticus* (Figure 3) Presence of lysis area around inoculated strains of tested cultures indicates their lysozyme activity.

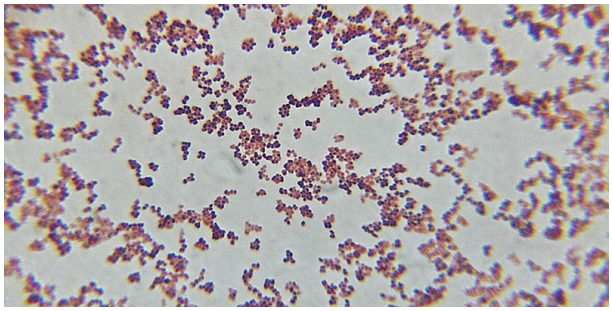

Fig. 3. *Micrococcus luteus var. Lysodeikticus*.

Antilysozyme activity of isolated *Serratiae* was determined according to Bukharin et al [3, 4, 9, 10, 13] by growth of micrococcus on Petri dishes with various content of lysozyme. This method allows determining qualitative and quantitative characteristic of antilysozyme activity in studied cultures.

Adhesive properties of tested cultures were identified by their ability to hemagglutinate sheep erythrocytes in a reaction with 3% of fresh sheep erythrocytes. D-mannose resistant hemagglutinating activity was determined by ability of serrated cultures to hemagglutinate sheep erythrocytes in presence of 1.5% of mannose [1, 9, 12].

Presence of hemolysine in tested *Serratia* cultures were determined by cultivation on 1.5% nutrient agar with addition of 3-5% of rabbit erythrocytes. In order to identify hemolysines, production of which depends on presence of thiol in blood agar, the blood agar was doped with 0.002% L-cysteine (thiol-dependent hemolysines) [5, 6, 9, 12, 13].

3 Results and discussion

Ability to lyse rabbit erythrocytes was recorded in 11 out of 19 cultures sourced form dairy products (amounting to 57.9%), in 15 strains isolated from goat, sheep and environmental samples and in 27 museum *Serratia* strains. Ability to hemagglutination in the presence of L-cysteine (thiol-dependent hemolytic activity) was found in 4 museum cultures of *Serratia* (6.6%), while no bacteria isolated from dairy products, sheep, goats or environmental objects demonstrated thiol-dependent hemolytic activity. (Table 1).

Table 1. Hemolytic activity in tested cultures of *Serratia marcescens*.

Property	Museum strains positive for the property		Strains sourced from sick animals and environmental objects, positive for the property		Strains sourced from dairy products, positive for the property		Total	
	Number of cultures	in %	Number of cultures	in %	Number of cultures	in %	Number of cultures	in %
Production of hemolysines	27	44.3	15	51.7	11	57.9	53	48.6
Production of thiol-dependent hemolysines	4	6.6	–				4	3.7

From studied 19 cultures of *Serratia* isolated from dairy products, ability for hemagglutination was shown for 14 strains, amounting to 73.7%; out of 61 museum strains, adhesive activity was identified for 45 cultures, i.e., 73.8%, while out of 29 cultures sourced from goats, sheep and environmental objects, there were 24 cultures positive for this property, amounting to 82.8%. Ability to agglutinate sheep erythrocytes independent of the presence of D-mannose was recorded for 8 strains out of 19 tested cultures sourced from dairy products (42,.1%), in 16 out of 29 cultures sourced from goats, sheep and environmental objects (55.2%) and in 35 out of 61 museum cultures (57.4) (Table 2).

No lysozyme-producing strains were produced out of *Serratia* cultures isolated from dairy products, while among 61 museum cultures 3 strains out of 29 produced lysozymes, amounting to 4.91%;

Table 2. Adhesive activity in tested cultures of *Serratia marcescens*.

Property	Museum strains positive for the property		Strains sourced from sick animals and environmental objects, positive for the property		Strains sourced from dairy products, positive for the property		Total	
	Number of cultures	%%	Number of cultures	in %	Number of cultures	in %	Number of cultures	in %
Adhesive activity in the absence of D-mannose	45	73.8	24	82.8	14	73.7	83	76.2
D-mannose-resistant adhesive activity	35	57.4	16	55.2	8	42.1	59	54.1

Out of samples sourced from goats, sheep and environmental objects, there were 2 strains positive for this property, amounting to 6.89%. (Table 3).

Table 3. Lysozyme and antilysozyme activity in tested cultures of *Serratia marcescens*.

Property	Museum strains positive for the property		Strains sourced from sick animals and environmental objects, positive for the property		Strains sourced from dairy products, positive for the property		Total	
	Number of cultures	in %	Number of cultures	in %	Number of cultures	in %	Number of cultures	in %
Ability to produce lysozyme	3	4.91	2	6.89	–	–	5	5.56
Antilysozyme activity	52	85.2	25	86.2	15	78.8	59	84.4

Ability to produce antilysozyme (Figure 4) was discovered in 15 out of 19 strains sourced from dairy products, amounting to 78.8%; among 61 museum strains, 52 strains demonstrated antilysozyme activity, amounting to 85.2%; out of 29 cultures sourced from goat, sheep and environmental objects, ability to produce antilysozyme was identified for 25 strains, that is, 86,2%. (Table 3). Quantitatively, by concentration of produced antilysozyme, the tests conducted on *Serratia* cultures showed that their antilysozyme activity varies in a range from 1 to 6 μg/ml.

Studies of lysozyme, antilysozyme, hemolytic and adhesive activity of Serratia on 61 museum strains, 19 strains isolated from home-made dairy products and 29 cultures sourced from goats, sheep and environmental objects allowed establishing that the stated factors are common among these bacteria. So, there were no strains found capable of producing lysozyme among 61 strains sourced from dairy products; out of 61 museum cultures, only 3 cultures produced lysozyme, that is, 4.91%, while out of 29 cultures isolated from goats,

4 Conclusion

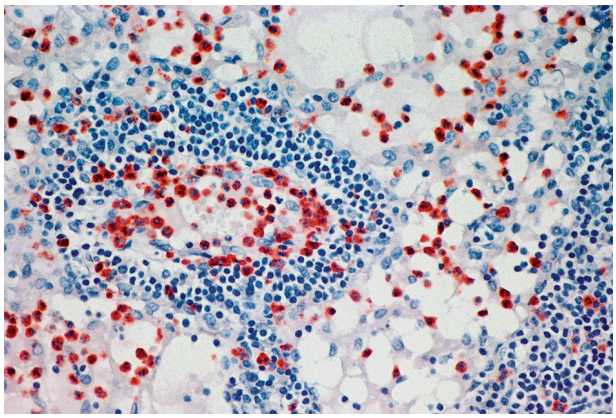

Fig. 4. Produced *antilysozymes*.

sheep and environmental samples, there were 2 strains positive for that property, amounting to 6.89%. Antilysozyme was produced by 15 cultures out of 19 isolated from dairy products (78.8%); some antilysozyme activity was demonstrated by 52 strains out of those 61 sourced from museum cultures; 25 out of 29 cultures sourced from goats, sheep and environmental samples were found capable of producing antilysozyme. Concentration of antilysozyme produced by various strains varied between 1 and 6 µg'ml.

Hemagglutination (adhesive activity) was found typical of 14 cultures isolated from dairy products (73.7%), 45 museum cultures (73.8) and 24 cultures isolated from goats, sheep and environmental objects (82,8%). D-mannose-resistant hemagglutination was detected for 8 strains (42.1%), isolated from dairy products, 16 strains (55,2%) sourced from goats, sheep and environmental objects and 35 museum strains (57.4).

Hemolytic activity with respect to rabbit erythrocytes were typical of 11 cultures (57.9%) sourced from dairy products, 15 cultures (51.7%) those sourced from goats, sheep and environmental objects and 27 museum strains (44.3%).

Thiol-dependent hemolytic activity was not detected for any tested strain of *Serratia* from dairy products or from goats, sheep and environmental objects. Among museum cultures, thiol-dependent hemolytic activity was typical of 4 strains, amounting to 6.6%.

References

1. R. A. Acharv, R. Kh. Gairabekov, F. S. Turlova, Z. E. Umieva, Adhesive ability of some opportunistic enterobacteria isolated from sheep with associated helminthosis and bacterial infection, *Natural Sciences* **2 (35),** 113–118 (2011)
2. O.V. Bukharin, N. V. Vasilyeva, B. Ia. Usviatsov, *Lysozyme of Microorganisms* (Tomsk, 1985)
3. O. V. Bukharin, B. Ia. Usvatsov, N. V. Nemtsova, Method for determination of antilysozyme activity of microorganisms, *Animal Microbiology* **2,** 27–29 (1984)
4. I. M. Gabrilovich, R. Kh. Gairabekov, T. Ch. Zhugova, K. Iu. Lotiev, O. S. Yakushenko, Lysozyme and antilysozyme activity of opportunistic enterobacteria, *Abstracts of the 18th All-Union Society of Epidemiologists, Microbiologists and Parasitologists named after I.I. Mechnikov* (Alma-Ata) pp 163–164 (1989)
5. I. M. Gabrilovich, R. Kh. Gairabekov, V. B. Boziev, M. V. Zarochentsev, Thiol-dependent hemolysines in opportunistic enterobacteria, *2nd All-Union Conference on Bacterial Toxins* (Yurmala) p 24 (1989)
6. I. M. Gabrilovich, R. Kh. Gairabekov, M. A. Sheozhev Thiol-dependent hemolysines as a factor of pathogenic power in *Citrobacter* and *Serratia, Materials of Bacterial Persistence Conference* (Kuibyshev) pp 69–71 (1990)
7. R. Kh. Gairabekova, M. R. Dakaeva, Pathogenic and opportunistic bacteria in milk and home-made dairy products, *Reflection* **1,** 19–23 (2019)
8. R. Kh. Gairabekova, M. R. Dakaeva, Pathogenic streprococci, staphylococci and opportunistic bacteria in milk and home-made dairy products, *Annals of Medical Institute* **15 (1),** 52–58 (2019)
9. R. Kh. Gairabekov, R. Kh. Gairabekova, Ia. A. Gaisumov, L. G. Molochaeva, F. S. Turlova Some peculiarities of epizootic processes accompanying enterobacterioses of goats and sheep in the territory of the Chechen Republic, *Reflection* **4,** 6–12 (2019)
10. R. Kh. Gairabekov, R. Kh. Gairabekova, S. A. Gubkhanova, F. S. Turlova, Z. E. Umieva Antilysozyme, anti-interferon and anti-complementary activity of some bacteria of family *Enterobacteriaceae, Modern problems of science and education* **5 (95),** 329 (2016)
11. R. Kh. Gairabekova Biological properties of *Serratia marcescens*, isolated from sheep in cases of post-dehelmintation disbacteriosis in the territory of the Chechen Republic, *Annals of Saratov State Agricultural University named after N.I. Vavilov* **4** 3–5 (2010)
12. 11 F. S. Turlova R. Kh. Gairabekov, Production of hemolysines by bacteria of genus *Serratia*, isolated from various sources in the territory of the Chechen Republic, *Annals of the Chechen State University* **1 (17),** 126–128 (2015)
13. 12 O. S. Yakushenko, A. B. Azamatova, V. V. Bugaeva, V. B. Boziev, K. Iu. Lotiev, R. Kh. Gairabekov, I. M. Gabrilovich, Factors of patogenic power of some opportunistic enterobacteria, *Materials of Conference Diagnostics, treatment and prevention of invasive and infectious diseases of agricultural animals* (Stavropol) pp 55–59 (1991)