

# Persistence Ability of *Citrobacter* Isolated from Dairy Products

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## ABSTRACT

A persistence ability is the capacity of pathogenic and opportunistic bacteria to survive in the aggressive internal environment of a macroorganism and is an important feature for these groups of microorganisms. The paper aims to establish whether the target *Citrobacter* cultures, once isolated from home-produced dairy products, have adhesive strength and hemolytic activity.

**Keywords:** persistence, bacteria, culture, strain, adhesion, hemolysin, thiol, agar, lysozyme, antilysozyme, hemagglutination.

## 1. INTRODUCTION

The ability of pathogenic and opportunistic microorganisms to remain viable under the aggressive internal environment of a macroorganism is a paramount necessary and important survival-driven attribute. This ability largely determines the pathogenic properties of these bacteria. Pathogenicity is a complex factor made up of a large number of microorganism abilities that are diverse in their effect on host organisms. Pathogenicity is an inherited species trait that is fixed in the genome of a phenotypic carrier, resulting from adaptation. It arises during the evolution of a parasite, i.e. it is a genotypic trait that indicates the capacity of a microorganism to penetrate into a macroorganism (infectivity) and multiply in it (invasiveness), causing the development of multiple pathological processes to occur in an infected organism during a disease.

The paper presents the findings showing the ability of *Citrobacter* cultures to hemagglutination and hemolysis. They were divided into two groups: the first group involved strains cultured from dairy products, the second group – cultures isolated from small ruminants.

## 2. MATERIAL AND METHODS

The paper attempts to identify the factors that allow *Citrobacter* to survive in the internal environment of

animals and humans, which is an aggressive environment for microorganisms. With this in view, 59 strains of *C. freundii* were tested once isolated from dairy products [9, 10] and 51 strains cultured from small ruminants to detect bacterial hemolysin and adhesive strength. 1.5 % beef-extract agar-agar (solid nutrient medium) was used to cultivate the target *C.* strains. 0.7 % beef-extract agar-agar (semi-liquid nutrient medium) was also used to create a second layer on top of solid agar. Incubation lasted 18–24 hours in a thermostat at 37 °C. Hemolytic abilities in the target bacteria were detected with nutrient agar supplemented with 3–5 % rabbit erythrocytes treble rinsed in Hanks solution [4–8, 11]. To identify hemolysins being produced subject to the thiol present in the blood agar, 0.002 % L-cysteine (thiol-dependent hemolysins) was added to the blood agar [2, 3].

The adhesive strength in *Citrobacter* was found in a 3 % blood cell hemagglutinin assay (red cells were freshly-received from a ram) in the presence and absence of D-mannose. The ability to agglutinate in the presence of D-mannose was considered as the presence of D-mannose-resistant adhesive activity in the target bacteria [4–8].

Lysozyme activity in the target *C.* cultures was evaluated on 1.5 % nutrient agar by the method of delayed antagonism after Bukharin et al. [1]. The cultures were incubated at 37 °C for 18–24 hours, then

the grown colonies were treated with chloroform vapors for 10 minutes, after which washed daily *Micrococcus luteus* var. *Lysodeikticus* cultures was twofold layered onto the target cultures in the dishes. A lysis zone found to be present around the inoculated strains of the target cultures signifies lysozyme activity.

The antilysozyme activity of *Citrobacter* isolated from various sources was determined by the method of Bukharin et al. [1] based on the micrococcal growth around the colonies of the target cultures on Petri dishes with different lysozyme content. The method determines the quantitative and qualitative productivity of antilysozyme activity in the target cultures.

**3. FINDINGS**

The tests to detect the *Citrobacter* ability to agglutinate sheep erythrocytes in the absence of D-mannose showed that 41 strains (69.5 %) out of 59 *C.* cultures isolated from dairy products had agglutinable capacity, so did 28 (54.9 %) out of 51 *C.* strains isolated from small ruminants (Table 1).

**Table 1.** *Citrobacter* ability for hemagglutination

Source of <i>C.</i> strains	No. strains	Hemagglutinating strains	
		Number	%
Dairy products	59	41	69.5
Ruminants	51	28	54.9

Laboratory tests aimed at establishing whether the *Citrobacter* cultures can agglutinate sheep erythrocytes in the presence of D-mannose (D-mannose-resistant hemagglutinating ability) identified that 4 strains (6.8 %) had this trait out of 59 *Citrobacter* strains cultured from dairy products and 6 cultures (11.76 %) out of 51 *Citrobacter* cultures isolated from small ruminants (Table 2).

**Table 2.** Ability for D-mannose-resistant hemagglutination among various *Citrobacter* cultures

Source of <i>C.</i> strains	No. strains	Hemagglutinating strains	
		Number	%
Dairy products	59	4	6.8
Ruminants	51	6	11.76

Besides, various *Citrobacter* cultures were tested for hemolytic activity. The tests showed that out of 59 *Citrobacter* strains isolated from dairy products 34 cultures (57.6 %) had the ability to lyse rabbit erythrocytes, and out of 51 *Citrobacter* strains cultured from small ruminants, this trait was found to be present in 30 cultures (58.8 %) (Table 3).

**Table 3.** Distribution of hemolytic activity among various *Citrobacter* cultures

Source of <i>C.</i> strains	No. strains	Hemolysin-producing strains	
		Number	%
Dairy products	59	34	57.6
Ruminants	51	30	58.8

The ability of the target *Citrobacter* cultures to produce hemolysins, whose secretion depends on thiol-containing components present in a nutrient medium – thiol-dependent hemolytic activity that was first described by Albesa et al. (1985) [2, 3] for *Klebsiella* cultures capable of thiol-dependent lysis of rabbit erythrocytes, made it possible to establish that these hemolysins were inherent in 7 strains out of 59 tested *Citrobacter* cultures isolated from dairy products, which amounted to 11.9 %, as can be seen from the data (Table 4). Fifteen strains had thiol-dependent hemolytic activity, amounting to 29.4 %, out of 51 *Citrobacter* cultures isolated from small ruminants on the territory of the Chechen Republic. (Table 4).

**Table 4.** Thiol-dependent hemolytic activity in various *Citrobacter* cultures

Source of <i>C.</i> strains	No. strains	Hemolysin-producing strains	
		Number	%
Dairy products	59	7	11.9
Ruminants	51	15	29.4

There were 13 strains secreting lysozyme out of 59 *Citrobacter* cultures isolated from dairy products, making up 22.03 %, while 8 strains were reported to produce lysozyme, making up 15.69 % out of 51 cultures isolated from small ruminants. (Table 5).

**Table 5.** Lysozyme activity in various *Citrobacter* cultures

Source of <i>C.</i> strains	No. strains	Hemolysin-producing strains	
		Number	%
Dairy products	59	13	22.03
Ruminants	51	8	15.69

The ability to produce antilysozyme was found to be present in 27 strains (45.76 %) out of 59 isolated from dairy products and tested cultures, whereas 39 strains (76.47 %) out of 51 cultures isolated from small ruminants were reported to have antilysozyme activity. (Table 6).

**Table 6.** Antilysozyme activity in various *Citrobacter* cultures

Source of <i>C.</i> strains	No. strains	Hemolysin-producing strains	
		Number	%
Dairy products	59	27	45.76
Ruminants	51	39	76.47

As shown by the tests, the quantitative exposure to antilysozyme (i.e. the concentration of produced antilysozyme) in the target *Citrobacter* cultures ranges from 1 to 6 µg/ml.

#### 4. DISCUSSION

Thus, the findings demonstrate that adhesive strength found to be present in the two groups of the target *Citrobacter* cultures isolated from dairy products and from small ruminants is a fairly common feature. Thus, 69.5 % of *Citrobacter* cultured from dairy products possessed this trait, and 54.9 % of strains among those isolated from small ruminants were reported to have this trait.

The D-mannose-resistant hemagglutinating ability identified in the target *Citrobacter* cultures made it possible to establish that this trait is not often found in these microorganisms. Thus, among the *Citrobacter* cultures isolated from dairy products, 4 cultures (6.8 %) were reported to have this trait, and among those cultured from small ruminants – 6 (11.76 %).

The ability to synthesize hemolysins is one of the significant properties for pathogenic and opportunistic microorganisms. Thus, the tests showed that out of 59 strains of *Citrobacter*, the ability to lyse rabbit erythrocytes was characteristic of 34 cultures (57.6 %), and of 51 strains of *Citrobacter* isolated from small ruminants, 30 cultures (58.8 %) had this trait.

The capacity of the target *Citrobacter* cultures to produce hemolysins, whose secretion depends on thiol-containing components present in a nutrient medium made it possible to establish that these hemolysins were inherent in 7 strains out of 59 tested *Citrobacter* strains cultured from dairy products, which amounted to 11.9 %, out of 51 *Citrobacter* cultures isolated from small ruminants, 15 strains were characterized by thiol-dependent hemolytic activity, i.e. 29.4 %.

There were 13 strains (22.03 %) secreting lysozyme out of 59 *Citrobacter* cultures isolated from dairy products, while 8 strains (15.69 %) were found to produce lysozyme, out of 51 cultures isolated from small ruminants. The capacity to produce antilysozyme was found to be present in 27 strains (45.76 %) out of 59 isolated from dairy products and in 39 strains (76.47 %) out of 51 cultures isolated from small ruminants.

#### 5. CONCLUSION

Adhesive strength in the two groups of *Citrobacter* cultures isolated from dairy products and from small ruminants is a fairly common trait. Thus, 69.5 % of the *Citrobacter* cultured from dairy products possessed adhesiveness, and among the strains isolated from small ruminants, 54.9 % were characterized by the presence of this trait. Thus, among the *Citrobacter* isolated from dairy products, 4 cultures (6.8 %) were characterized by D-mannose-resistant hemagglutinating ability, and among those cultured from small ruminants – 6 (11.76 %).

The ability to lyse rabbit erythrocytes was characteristic of 34 strains (57.6 %) cultured from dairy products, and 30 cultures of 51 *Citrobacter* strains isolated from small ruminants (58.8 %). Thiol-dependent hemolysins were inherent in 7 strains from 59 target *Citrobacter* strains cultured from dairy products, which amounted to 11.9 %, and 15 strains from 51 *Citrobacter* cultures isolated from small ruminants, i.e. 29.4 %.

There were 13 strains (22.03 %) secreting lysozyme out of 59 *Citrobacter* cultures isolated from dairy products, and among 51 cultures isolated from small ruminants, 8 strains (15.69 %) produced lysozyme. The ability to produce antilysozyme was found to be present in 27 strains (45.76 %) out of 59 isolated from dairy products and tested cultures, and in 39 strains (76.47 %) out of 51 cultures isolated from small ruminants.

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