LYSOGENY AND LYSOSENSITIVITY IN SALMONELLA STRAINS

Nicolae Mocuța¹, Gheorghe Ștețca², Vasile Chindriș³, Adriana David^{3*}

¹Food Safety Laboratory SC COMPPIL SRL Zalău; ²DSVSA Salaj; ³University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca; *Corresponding author: adriana.david@usamvcluj.ro

Abstract. Bacteriophages are viruses pathogenic to bacteria, that is, "bacteria eaters". The results of the activity of the phage to bacterial cells manifests itself through the following phenomena: (a) The bacterial cell is lysosensitive (it allows the phage to multiply, with bacterial lysis); (b) It is Lysogenic (it carries the bacteriophage in its genome and transmits it from generation to generation); (c) It is lysoresistant (it has no receptors for bacteriophages and resists to the attack of the phage). Though once believed the bacteriophages would become a universal treatment for bacterial disease, this has not proved valid from reasons like inactivation within the organism or the emergence of resistant bacteria. However, as antibioresistence is becoming more common and as the importance of establishing the filiation of the strains involved in food poisonings, the importance of bacteriophages has become actual again. To carry out this study, liquid and solid culture media has been used, young cultures of the researched strains, Petri dishes, and sets of bacteriophages obtained from lysogenic cultures. For studying lysogeny the cultures were seeded onto solid media in squares drawn on the dish and after the culture dried, drops of the liquid media culture that was surveyed for lysogeny were added. After 24h at 37C, we read the results - with clear evidence of areas of cellular lysis. To check for lysosensitivity the tested cultures were seeded onto Petri dishes and after drving the prepared phages were added. After incubation, we checked the cellular lysis areas. The findings are illustrated in photo 1 (lysogeny) and photo 2 (lysosensitivity). After completion of the tests it was revealed that a number of 6 Salmonella strains of the 45 in total (13.33%) were lysogenic and a number of 18 strains (40%) were lysosensitive. From current literature, it is evident that isolation of bacteriophages and their standardization and grouping in sets for phagic profiling has become of great importance as the method of phagic profiling is very quick, easy, and cheap when compared to genetic, biochemical, and serologic methods - and can be used complementary, with other sets of bacteriological diagnosis.

Keywords: lysosensitiv, lysogeny, strains, Salmonella

INTRODUCTION

Observations on the phenomenon of bacteriolysis were made long ago. In 1896 Hankin noticed that river water had lithic properties for choleric vibrions, but the phenomenon was explained in 1915 by the Twort, which called it "transmissible lysis." In 1917, Herelle, introduces in the literature the term bacteriophage, that is, a "bacteria eater", and Bordet and Ciuca discover the phenomenon of lysogeny in E. coli. From the morphological point of view, the bacteriophage consists of the head, the tail and an intermediate disc. The head contains the genome, double-stranded DNA, protected by capsid, and the tail is made up of proteins and features a cylindrical tube called the axial cylinder. The result of phage activity on bacterial cells is manifested by the following phenomena: a) the bacterial cell is lysosensible (it has bacteriophage as a profag in its genome and transmits it From generation to generation) c) is lysoresistant (has no bacteriophage receptors and resists bacteriophage attack.

Lysogeneic bacteria may become lyoresisable, and can not be attacked by other homologous bacteriophages. Bacteria infected with a beech may present some missing characters to non-infected cells, called phenomenon being called phagic conversion.

Depending on the cycles of development phages may be temperate phages and virulent phages, which always produce cell lysis after their penetration into cells. During the replication of moderate phage in the viral particle, bacterial DNA fragments can be introduced which can then be introduced into the DNA of other bacteria that have resulted in a genetic transfer, a process called phage transduction.

MATERIAL AND METHOD

A very important aspect is that the lytic activity of bacteriophages manifests only on lysosensitive cells and can be evidenced by cultivation on solid or liquid culture media. Practically cultivation of the strains studied for lysosensitivity on culture media to which is added Lysogenic strains or bacteriophages obtained by filtration from lysogenic strains. If the strain is lysosensible, this can be seen by clarifying the liquid medium or obtaining clear areas, so-called well-defined lysis beads, on solid media. Since bacteriophage lysis activity is very specific for bacterial strains, it is used in bacteriology and especially in epidemiology as the basis for phagic lymphoptitis and determining the origin of bacteria involved in food poisoning.

The following materials were used to perform the research:

- Liquid and solid culture nutrients.
- Young crops on liquid and solid media (24h) of strains to be researched,
- Sets of bacteriophages previously prepared from lysogenic culture.

The consistency of the phages is long-lasting by cold storage (12 years, Bordet) or even more by lyophilization.

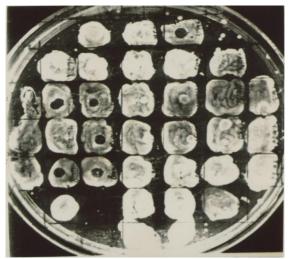


Fig. 1. Lysogeni of Salmonella strain

Working mode: The Petri box were prepared by penetrating 1.5 cm of squares (as shown in picture no. 1), after which the molten agar was poured into the plate and allowed to solidify to cool, then strain examined for lysosensibility by flooding the plate, let it dry with the half-open cap and then, sowed with an ansa, a drop of strains investigated for

lysogeny, culture 24 hours each The plates were allowed to dry, with the caps half-open and incubated at the thermostat at 37 degrees Celsius for 24 hours. They read the results. As can be seen in picture no.1, a number Of 6 strains have been found to be lysogenic, and clearly distinguishable clear rounded beaches can be clearly distinguished as a result of the lytic activity of the bacterial strains grown in the respective squares.

Multiplication of bacteriophages was accomplished by cultivating portions of lysate broth agar in broth and after incubation for 24 hours at 37 degrees Celsius. Centrifuged. The supernatant was separated and stored in a refrigerator or freezer.

Using this procedure, a number of 16 antisalmonella bacteriophages were isolated. Using these phages, the phagic typing of some Salmonella strains in the laboratory was attempted. Figure 2 illustrates the lysosensitivity of a Salmonella strain to the laboratory isolate of the F-13 bacteriophage, through the presence of the two well-known lizards. There are several methods of isolation and testing of bacteriophages: isolation by sowing enrichment method, OTTO method, FURT method, FISHER method.

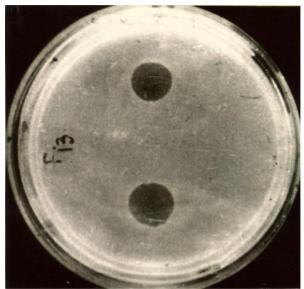


Fig. 2. Lysosensitivity of a Salmonella strain

RESULTS AND DISCUSSIONS

Reading the results of the lysogene and lysotype tests resulted in a total of sixteen strains (13.33%) of the 45 strains of Salmonella examined, which were lysogenic (carriers of bacteriophages) and a number of 18 (40%) s They turned out to be lysosensitive. (3) Internationally and in our country have been isolated and standardized sets of bacteriophages for different bacterial species that can be used both for the detection of bacterial species as well as for traceability of food contamination.

For example, for staphylococcal phagocytosis in our country, group I phage was used 29,52A, 72,80 group II 3A, 3C, 55,71, group III 6,42E, 47,53,54,75,77, 83A, 84.85 (1).

Conclusions. Although it has been hoped that the bacteriophage will be a universal drug with which all microbial diseases can be treated, the results are not at the level of expectations, primarily because the phages are inactivated in the digestive tract, but lizo

stems -reflective, rapid elimination of the phage in the body, and its inactivation by the serum and the appearance of anti-phage antibodies. However, it has not been entirely discontinued especially in local treatments and due to their total lack of harm. Due to the specificity of their action Phagas are used in epidemiology to establish the traceability and origin of lizotypic infections. It is also important to study the bacterial-phage-gene relationship as the best genetic study model. In practice, the phage interferes with a series of industrial processes and lysogenesis of the strains Bacteria used in fermentation (dairy products, food) in the antibiotic industry presents a great deal It is important practice. Bacteriophage can be a pathogenic factor and is an agent of bacterial variability. In principle, many strains of Salmonella have been shown to be lysogenic, thus bearing bacteriophages integrated into their own chromosomes and lysosensitive, i.e. lysates of bacteriophages.

Knowing the importance of using bacteriophages in these cases, we believe that further research is needed in this area and the expansion of research into other bacterial species.

REFERENCES

- 1. Dumitru Buiuc, Marian Neguț. 2017, Tratat de microbiologie clinică. Ediția a III-a –Editura Medicala.
- 2. Dobre Barzoi, Sergiu Meica, Marian Negut. 1999, Toxiinfectiile Alimentare ed. Diacon Coresi (Bucuresti 1999).
- 3. M. Decun, Un punct de vedere cu privire la riscurile contaminării cu E. COLI ... https://www.lumeasatului.ro/.../2757-un-punct-de-vedere-cu-privire-la-riscurile-conta, 9 mar. 2016
- 4. Nicolae Mocuta, Teza de doctorat USAMV Timisoara 1994.
- 5. Ordeanu V., Curs de microbiologie generala si farmaceutica » UMF,FF an II, 2009 (nepublicat).
- 6. Microbiologie Bacteriofagul T4, Scribd https://ro.scribd.com/doc/160416172/Microbiologie-Bacteriofagul-T4/10.07.2017