ISOLATION OF BACTERIOPHAGES FOR BIOCONTROL OF PATHOGENIC MEAT BACTERIA

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I. INTRODUCTION

Contamination of meat products can occur mainly due to the presence of microorganisms belonging to the natural microbiota of animals intended for consumption. These microorganisms can compromise the microbiological quality and safety of meat products by system failures or abuses during food animal production, product processing and distribution, and preparation for consumption, as well as by consumption habits [1]. It is essential to promote research and development of techniques to guarantee the safety of meat products, in relation to the presence of chemical residues harmful to the human body and pathogenic microorganisms, including, the control of resistant bacteria to chemical agents that can cause damage throughout the production chain. In this scenario, a promising possibility arises through the use of bacteriophages or phages, which are viruses that can act in the biocontrol of pathogenic bacteria. Phages have interesting properties for the food industry because they specifically infect the bacterial host, replicate in the presence of this host, and are widely distributed in the environment, as well as in many types of food [2]. Phages can be used prophylactically, therapeutically and to sanitize surfaces and for spraying on meat products or packaging. Unlike antibiotics, they offer greater versatility in choosing phage cocktails and treatments to be used in the meat production chain. Thus, due to the great advantages of using biocontrol through phages, its application in meat products is a promising and valuable tool to help control undesirable microorganisms present in products of animal origin.
This work aims to isolate bacteriophages with inhibitory action on relevant pathogenic bacteria which can be found in meat and meat products.

II. MATERIALS AND METHODS

The bacterial strains used in this study belong to the CTC – ITAL laboratory collection and consisted of type strains and environmental sources isolates of *Listeria monocytogenes, Salmonella typhimurium*, *Escherichia coli* O157:H7, and *Staphylococcus aureus*. Swab samples from contact surfaces were collected from the pipelines of the sewer and drains of the meat processing plant of CTC-ITAL, following standard methods of identification. Bacterial strains were routinely grown in TSB culture medium incubated at 37°C, and permanent stocks were stored at -80°C in 20% glycerol.
Phages were isolated through concentration with polyethylene glycol, according to [3]. For this, samples (500ml) of the environment in which animals are raised (soil from the cattle corral and lagoon containing pig waste) from the city of Pirassununga, SP, and from the sewage of a local meat processing plant from CTC/Ital, were analyzed. The samples received SM buffer and NaCl to the final concentration of 1M and were centrifuged (7,500rpm) to remove impurities. The supernatant received Polyethylene Glycol 8000 to a final concentration of 10% (w/v) and was kept at 4°C for a overnight decantation. The material was subjected to centrifugation at 7,500rpm for 20min, the pellet was resuspended in SM buffer and extracted with an equal volume of chloroform. The tubes were centrifuged at 7,500rpm for 10min and the supernatant containing the phage extract was stored at 4°C protected from light. Phages were isolated through the agar overlay method [4]. Single lysis plaques were collected with sterile picks, and added to an exponential growing bacterial culture following incubation overnight at the suitable temperature.
For the induction of bacteriophage infection, 200µL of active bacterial cultures were transferred to sterile 2mL microtubes. 300µL of phage extract was added to these tubes followed by incubation at 30°C for approximately 1h. After this period, tubes containing 5mL of semi-solid culture medium (0.7% agar) were melt, which were kept in a water bath at 44°C. Then, the bacterial suspension and phage extract were transferred to semi-solid culture medium tubes, gently homogenized and evenly distributed over the

surface of Petri dishes containing the appropriate culture media. After the media solidified, the plates were incubated at the optimum growth temperature for each microorganism for 24h and the formation of lysis plaques was evaluated.

III. RESULTS AND DISCUSSION

From the swabs collected at the meat processing plant, six strains of *L. monocytogenes* were isolated, which were included as indicators in the tests along with the type cultures. The inhibitory action of the phages could be evidenced by the formation of clear areas on the plates inoculated with *E. coli* O157:H7 ATCC 43895 or *L. monocytogenes* ATCC 7644 (Figure 1). Regarding *Staph. aureus* and *S. typhimurium*, no phages with inhibitory action on these bacteria were isolated from the samples tested. The isolated viral extracts were coded as phages A4, A7, A14, A15, and A17 and were detected in samples collected from animal breeding environment and lagoon water. *L. monocytogenes* was infected by three phages (A4, A7, and A14), while *E. coli* O157:H7 was infected by all the phages isolated. Therefore, phages A4, A7 and A14 presented an unusual trait in relation to the other phages, showing inhibitory action on both bacterial cultures. On the other hand, phages A15 and A17 showed specificity only for *E. coli* O157:H7. According to Nikolich & Filippov [5], phages infect their bacterial hosts in a specific way, and it is more common for phages to infect only one species of bacterium or a subgroup within the same bacterial species. Thus, it is important to investigate the reason for the cross-reactivity between two different genera of bacteria and these divergent phages, clarifying whether this is a real property or just a consequence of testing a phage mixture that has not yet been purified.

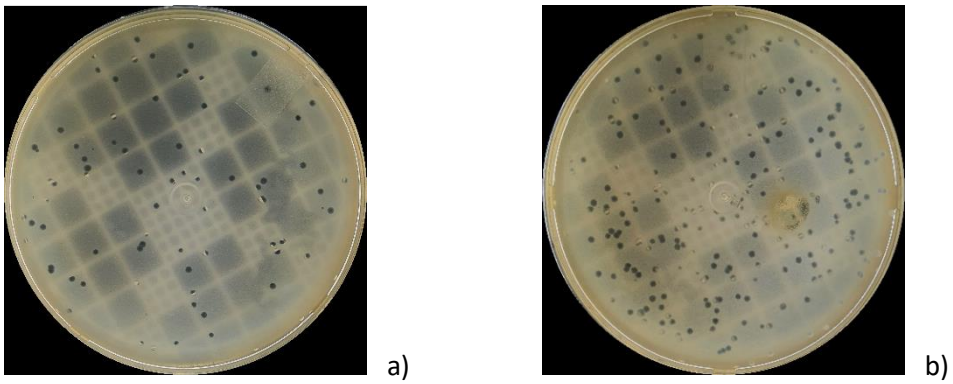

Figure 1. Agar plates with phage A7 with a) *E. coli* O157:H7; b) *L. monocytogenes.*

IV. CONCLUSION

Phages with inhibitory potential against *L. monocytogenes* and *E. coli* O157:H7 were detected, but it was not possible to obtain phages with action against *Staph. aureus* and *S. typhimurium*. It was demonstrated that isolated phages can be efficient in controlling the growth of bacteria which are harmful to the meat industry.

ACKNOWLEDGEMENTS

The authors thank CNPq for the scholarship.

REFERENCES

1. Sofos, J.N. (2014). Food safety management. A practical guide for the food industry. In Motarjemi, Y., Lelieveld, H., Meat and Meat Products (pp 119-162). Amsterdam: Elsevier.
2. Kennedy, J.E., Wei, C.I., Oblinger, J.L. (1986) Distribution of coliphages in various foods. Journal of Food Protection, 49: 944-951.
3. Gregoracci, G.B. (2006). Levantamento de bacteriófagos líticos: isolamento e caracterização de vírus provenientes de esgoto comum com potencial aplicação antimicrobiana. 2006. 101 f. Tese (Doutorado) – Universidade Estadual de Campinas, SP.
4. Carlson K. (2005). Working with bacteriophages: Common techniques and methodological approaches. In Kutter, E., Sulakvelidze, A., Bacteriophages*:* Biology and Applications (437-494). Boca Raton: CRC Press.
5. Nikolich, M.P., Filippov, A.A. (2020). Bacteriophage Therapy: Developments and Directions. Antibiotics, 9: 135.