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Genomic analysis and morphological characterization of bacteriophages for Salmonella spp. treatment in poultry

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Abstract
Enteric infections caused by Salmonella spp. represent a major public health problem worldwide, due to the large proportion of foodborne infections derived from this pathogen. Currently, antimicrobials are used to prevent contamination of chicken meat. However, in order to combat salmonellosis without the propagation of resistant strains, it is necessary to study alternative therapeutic approaches, such as the use of bacteriophages against Salmonellosis. For the present work bacteriophages provided by FMRP-USP were selected to further studies on its therapeutic potential. In addition, we work with lytic bacteriophages induced from monophasic strains of Salmonella spp. Our initial aim for this project was the morphological and molecular characterization of these viruses. Nevertheless, the environmental phages did not survive the storage period. Due to these results, our further studies will be focused on the lytic phages. They will be tested in vivo in the C. elegans model in order to evaluate the survival rate of the worms when infected with Salmonella spp.

Key words: *Salmonella spp., Bacteriophages, Poultry*

Introduction

One of the most common foodborne diseases is salmonellosis, an infection caused by Salmonella spp. a genus of bacteria. This infection not only affects humans but also jeopardizes the poultry industry. Due to the high demand for poultry products worldwide, use of antimicrobials as growth promoters in chicken has been a common practice. However, with the increasing number of resistant bacteria, it is necessary to invest in alternatives therapies to avoid foodborne pathogens. One of the solutions can be the use of bacteriophages therapy.

Bacteriophages are viruses that infect and lyse bacterial cells. They are widely distributed in the environment. Phage therapy has been used to treat and prevent enterobacteria infections in animals. This approach has a great potential to substitute antimicrobials as growth promoters. The present work consists in studying the therapeutic potential of phages against salmonellosis. We have studied phages isolate from chicken farms and induced phages of monophasic Salmonella spp.

Results and Discussion

For this study, 9 bacteriophages were selected from a previous research conducted at FMRP-USP. Furthermore, we also worked with two bacteriophages: Saeni 691 and Saeni 633. Both were previously studied in our laboratory. They are lytic phages induced in S. enterica I,4,[5],12:i:- and Salmonella Typhimurium respectively. They belong to Caudovirales order and Podoviridae family. Mytomicin 2% was used to induce lytic phase.

In order to recover the bacteriophages, the Double-layer Agar methodology was conducted as described in Image 01 (A). The environmental phages could not be recovered, therefore the morphological and genotypic characterization were not performed.

Our group intends to conduct the therapeutic potential of the induced lytic phages testing the C. elegans model as described in Image 01 (B). Worms will be fed with E. coli OP50 and SE 618, therefore the cultures will be infected with phages. The lifespan of worms will be compared.

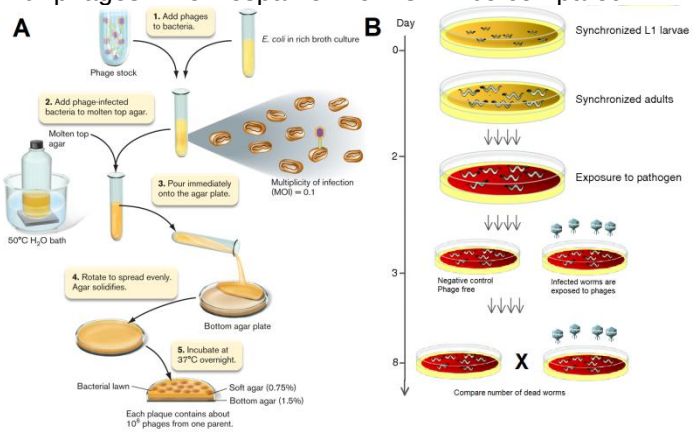

Image 1. Representation of the mainly methodologies used. A) The double-layer agar method.[1] B) Infection of *C. elegans* with bacteriophages[2].

Conclusions

The unsuccessful trials on phages recovery worked as indicative of its instability, which is a characteristic that cannot be present in therapy phages. Most likely the viruses have lost infectivity due to the long-time storage.

Phages Saeni 633 and Saeni 691 are still being tested, therefore we do not have any conclusive data. Nevertheless, we expect to see an improvement of *C. elegans* lifespan treated with the phages comparing with the ones not treated.

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