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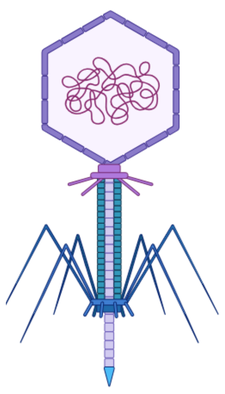
UTA RAM
ONE HEALTH
Antibiotic Resistance & Phage

Lytic bacteriophages against multi-resistant *Salmonella enterica* from avian origins in Tungurahua province

Paulina Topa Pila[1], Katheryne Morales Cunalata[1], William Calero Cáceres[2]

These authors contributed equally to this work and share co-first authorship 1. UTA-RAM-One Health, Department of Food and Biotechnology Science and Engineering, Universidad Técnica de Ambato, Ambato, Ecuador 2.

INTRODUCTION



Salmonella enterica is one of the most common bacteria involved in cases of foodborne infections and intoxications in humans, with chicken being its main transmission source worldwide. Antibiotics have been used to control this problem, however, their excessive use has led to the emergence of multi-resistant strains of *Salmonella*. Therefore, the use of lytic bacteriophages has emerged as a new biocontrol strategy, whose lytic capacity is being applied to eliminate pathogens in raw and ready-to-eat foods. This project focuses on isolating lytic bacteriophages for multi-resistant *S. enterica* of avian origin from the province of Tungurahua, and their evaluation according to lytic profiles and stability.

MATERIALS AND METHODS

The host bacteria were selected based on sensitivity determined by the Kirby-Bauer test and molecular genotyping via (GTG)5- PCR.

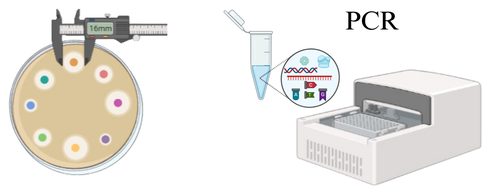


Figure 1. Selection of *Salmonella* hosts

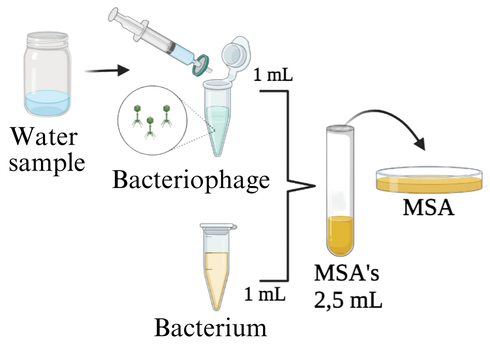


Figure 2. Isolation of bacteriophages

Bacteriophages were isolated and purified using the double-layer agar method and Modified Scholten Medium (MS), deploying two strains of *S. enterica* serovar Infantis. Post-purification, the lytic profiles of bacteriophages were evaluated through the spot test.

The stability of bacteriophages was evaluated at a cooling temperature of 4 °C, freezing temperature of -18 °C, and at pH 5.5 and 7.2. There were a total of 4 experiments (A, B, C, D) for each phage.

Experiments	Temperature °C	pH
A	4	5.5
B	4	7.2
C	-18	5.5
D	-18	7.2

Table 1. Experiments

CONCLUSIONS

A total of 27 bacteriophages were isolated and purified for *S. enterica*, isolated from contaminated river water sources in the provinces of Tungurahua and Cotopaxi, using two strains of multidrug-resistant *S. enterica* serovar Infantis of avian origin (S2 and S9V23). The evaluation of their lytic profiles and stability at low temperatures demonstrated their potential as biocontrol agents for pathogenic microorganisms applied to raw foods such as chicken.

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RESULTS AND DISCUSSION

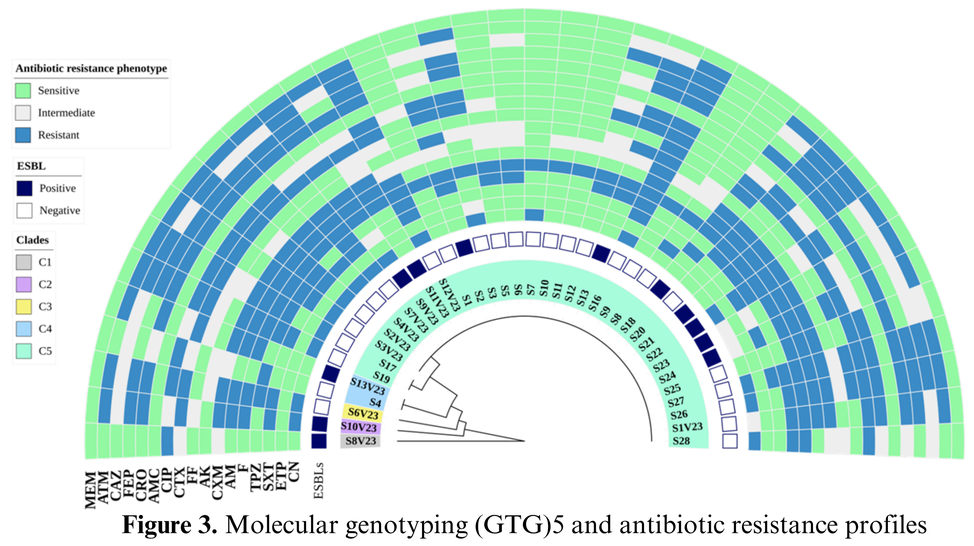

Figure 3. Molecular genotyping (GTG)5 and antibiotic resistance profiles

The 38 strains were identified as *Salmonella enterica*, of which 37 belonged to the Infantis serovar. (GTG)5-PCR allowed grouping the isolates into 5 clades, showing very little genetic variability. The analysis of resistance profiles showed that 73.68% of the strains (28/38) exhibited multidrug resistance (MDR), of which 12 were producers of ESBL. Taking into account the data, strains S2 and S9V23 were chosen as hosts.

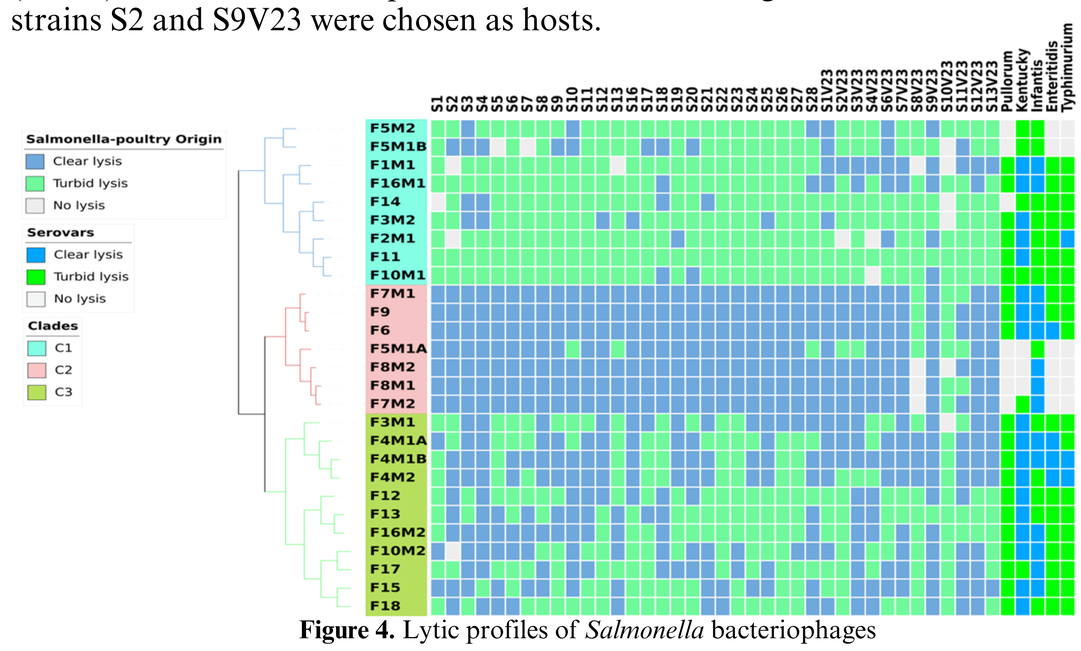

Figure 4. Lytic profiles of *Salmonella* bacteriophages

A total of 27 bacteriophages were isolated. The evaluation of their lytic profiles allowed grouping the phages into 3 clades, according to their host range, and based on this, phages F6 F7M1 and F4M1B were chosen for stability evaluations.

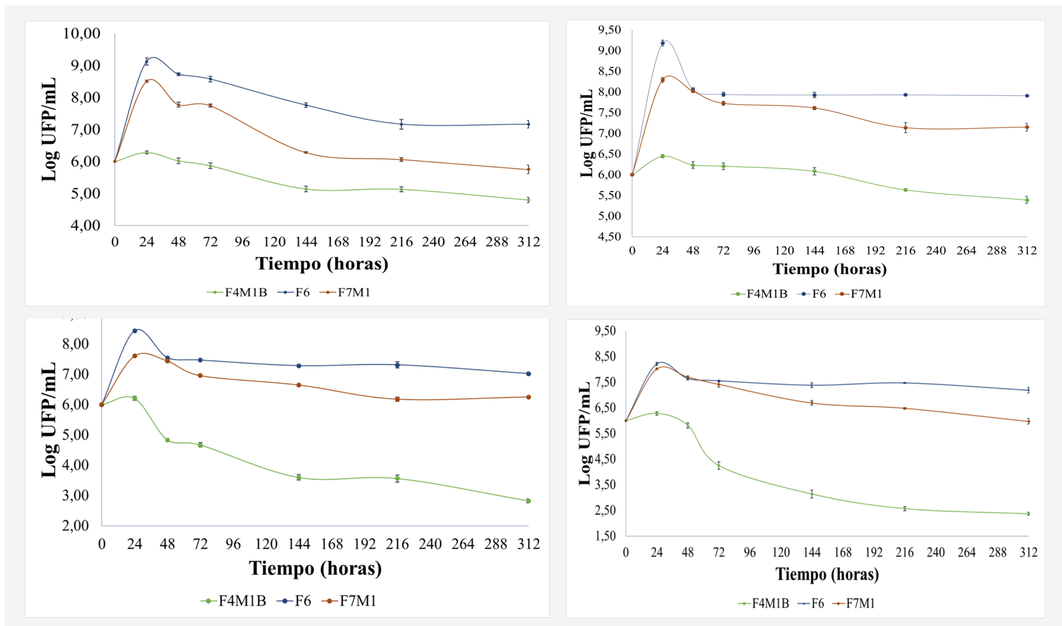

Figure 5. Stability tests of bacteriophages F6, F7M1, and F4M1B

The F6 phage remained viable in all four experiments for 312 hours, managing to inactivate the bacteria in 216 hours at a temperature of -18 °C and pH 7.2. The F7M1 phage showed a significant reduction at a temperature of 4 °C and pH 5.5, but not at a temperature of -18 °C where the pH effect was not significant. The F4M1B phage maintained its activity at a temperature of 4 °C without significant pH influence, but lost its activity at -18 °C after 216 hours.

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