

Isolation, and biological and molecular characterization of specific bacteriophages against *Klebsiella pneumoniae* and *Serratia marcescens* from hospital wastewater and WWTP of Panama

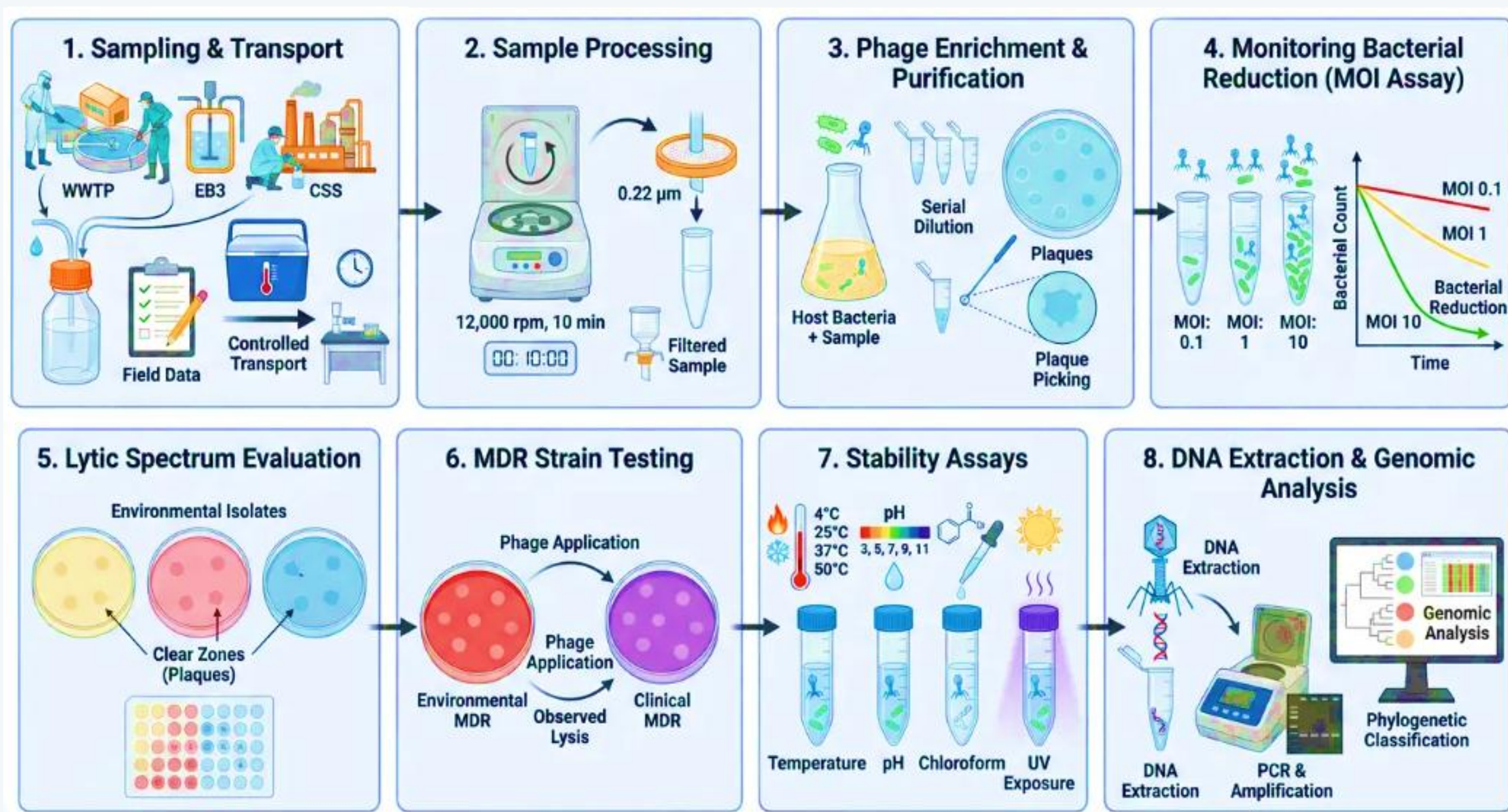
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Introduction

Antimicrobial resistance is a major global public health threat, renewing interest in bacteriophages as therapeutic alternatives. These viruses specifically infect bacteria and are harmless to humans. Phage therapy is particularly relevant against WHO-critical priority pathogens such as *Klebsiella pneumoniae* and *Serratia marcescens*, which frequently cause nosocomial infections. As antibiotics become less effective, the selective use of bacteriophages offers a promising strategy for controlling multidrug-resistant infections and improving patient outcomes in modern healthcare systems worldwide. However, the main limitation of phage therapy is the lack of well-characterized libraries targeting specific pathogens in each location.

Methodology



3. MOI determination in host bacterial strains of Bacteriophages

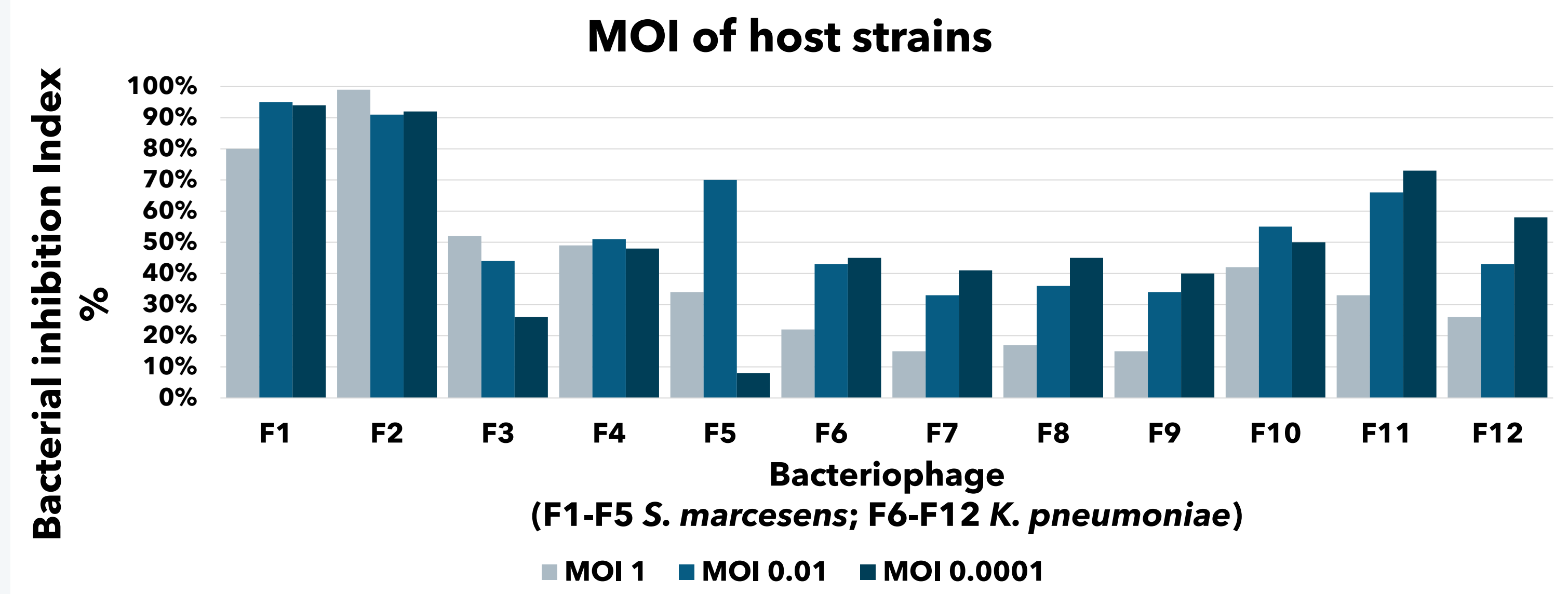


Figure 4. Lytic inhibition indices of bacteriophages against hosts over 24 h at MOIs of 1, 0.01, and 0.0001.

4. Physicochemical Stability

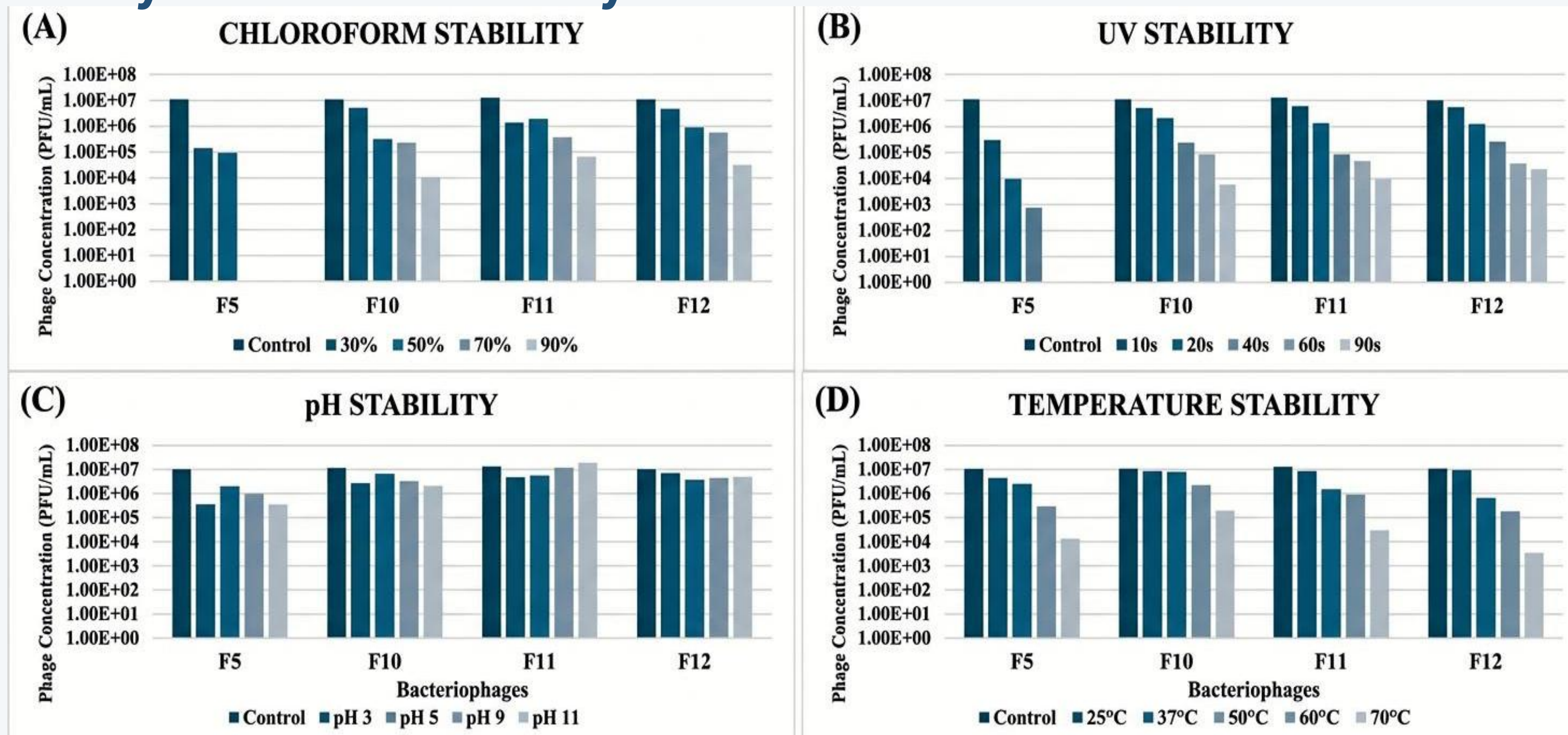


Figure 5. Biological characterization of bacteriophages F5, F10, F11, F12. Concentration (UFP/mL) as a function of: **A)** Chloroform (0 - 90%); **B)** Exposure to UV light (0 - 90 s); **C)** pH (3 - 11); **D)** Temperature (25-70°C).

Results

1. Isolation of Bacteriophages

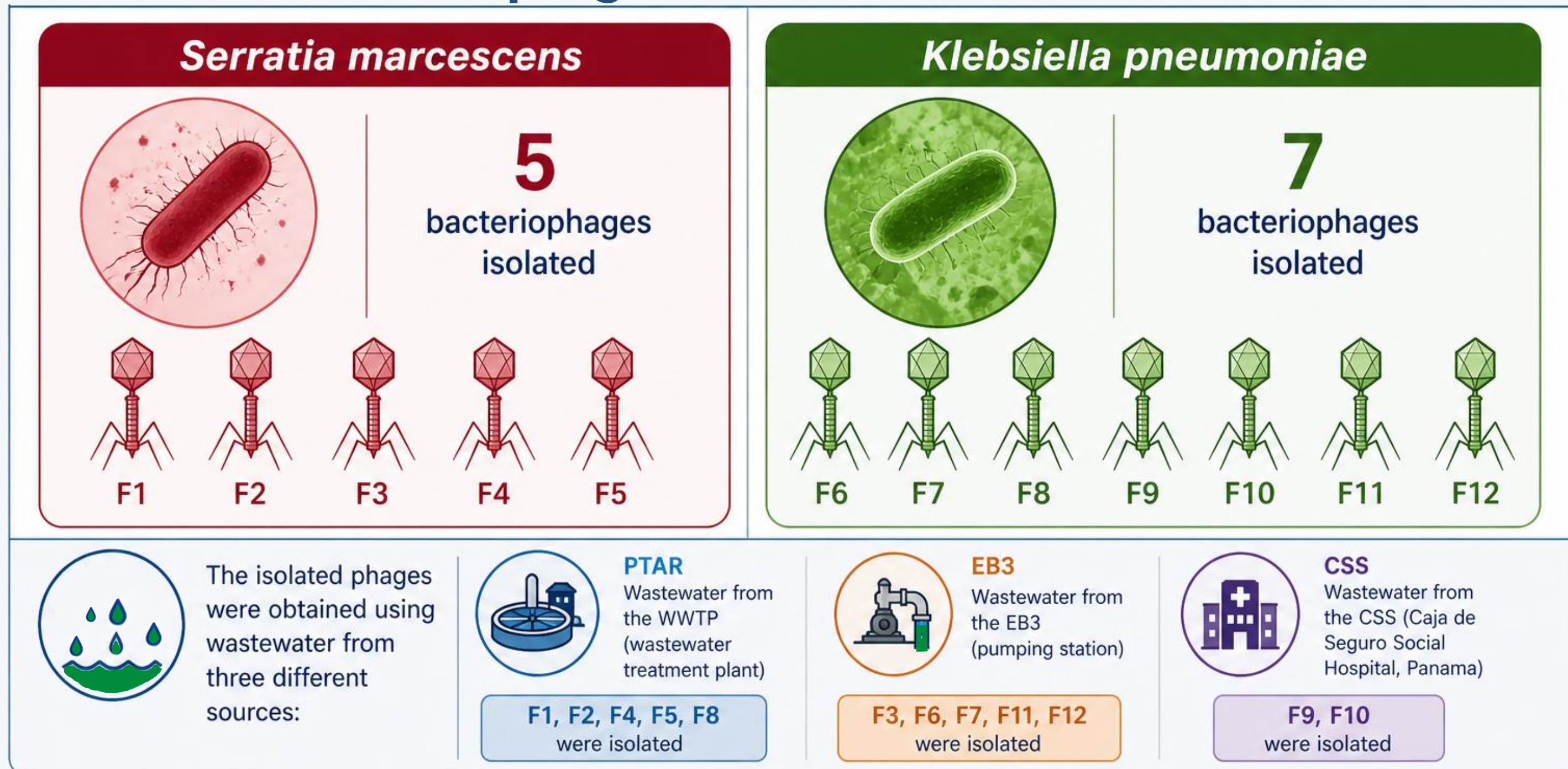


Figure 1. Summary of the isolation and purification of *K. pneumoniae* and *S. marcescens* bacteriophages, categorized by sampling site.

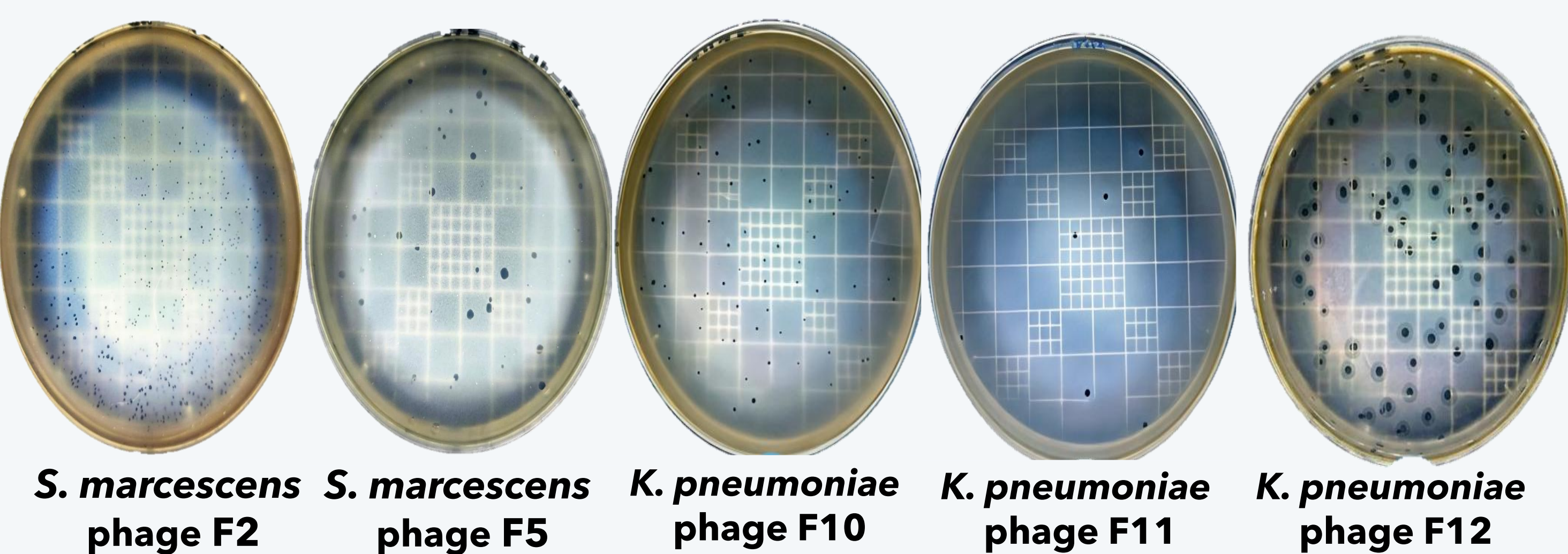


Figure 2. Morphology of phage plaques on TSA double-layer agar.

2. Host Range

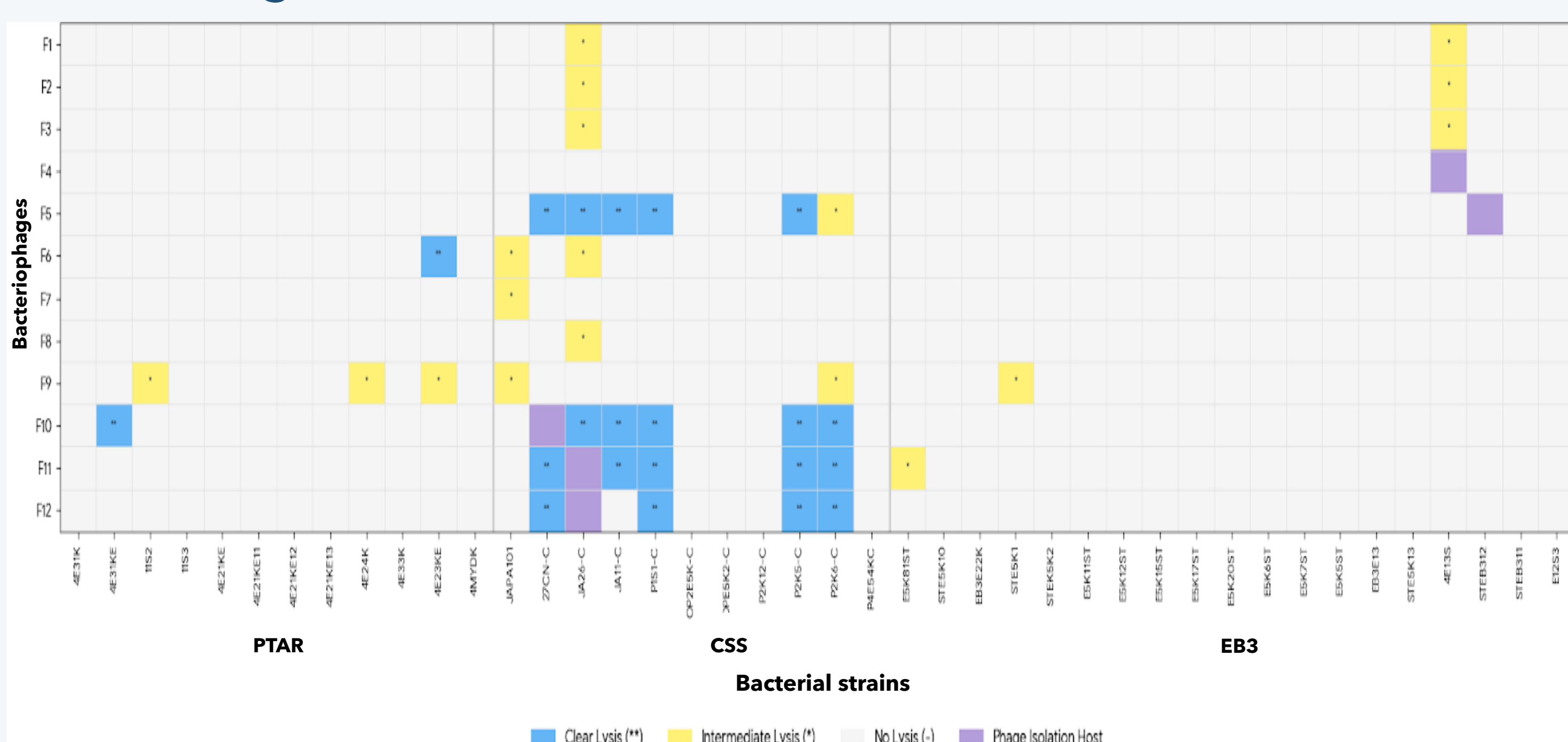


Figure 3. Host range of bacteriophages against *K. pneumoniae* and *S. marcescens* strains isolated from PTAR (WWTP), EB3, and CSS wastewater. **Sky blue:** clear lysis; **yellow:** intermediate lysis; **grey:** no lysis; **purple:** isolation host.

5. Genomic Analysis

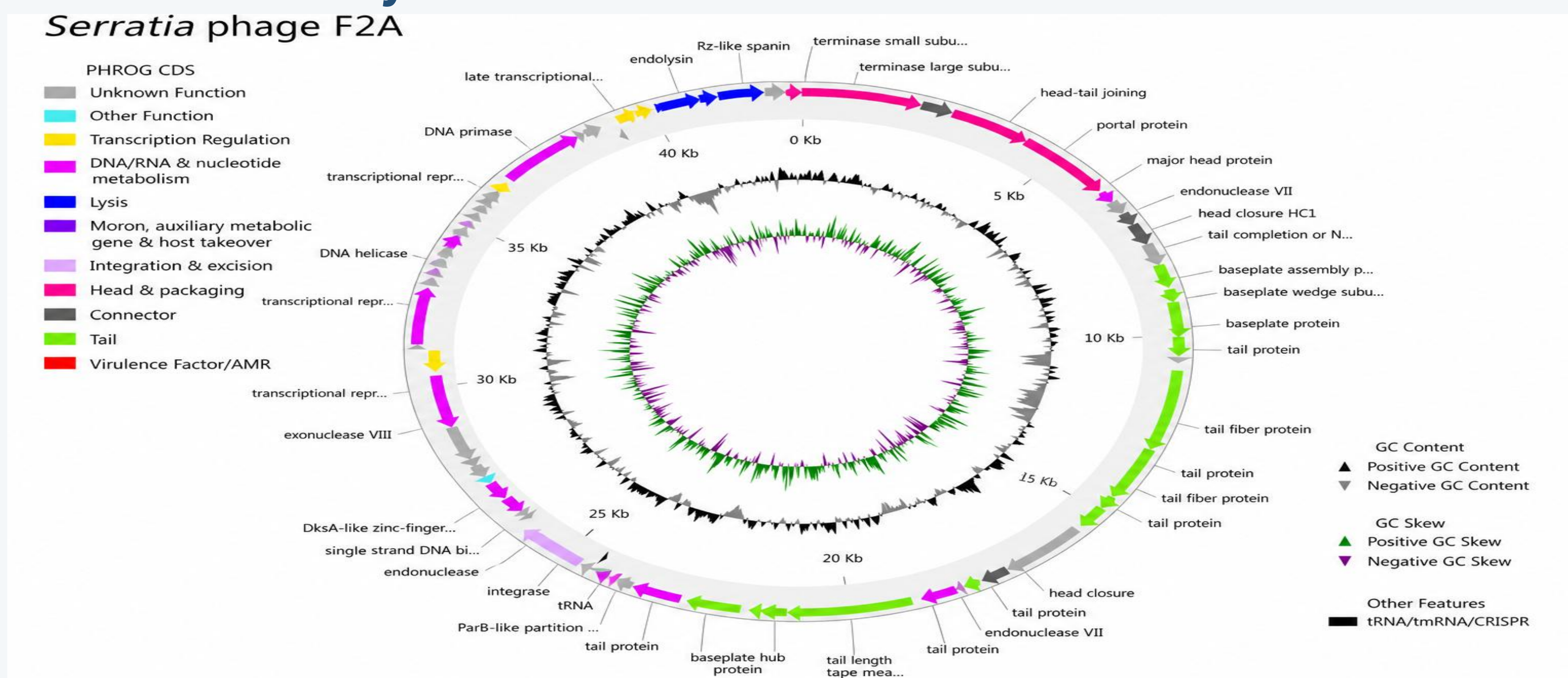


Figure 6. Circular map of the *S. marcescens* phage F2 genome, annotated with Pharokka. The genome is considered safe as it lacks genes associated with virulence factors or antibiotic resistance.

Conclusion

- Phages **F10**, **F11**, and **F12** exhibited the broadest host range and superior lytic efficiency, particularly against MDR clinical isolates from hospital environments (CSS). These phages maintained high titers across a broad pH range (**3-11**) and showed thermal tolerance up to 60 °C. Furthermore, they remained viable after prolonged UV exposure (**90s**) and exposure to high concentrations of chloroform (**90%**).
- Phage **F5** demonstrated significant **cross-reactivity** between *K. pneumoniae* and *S. marcescens*, coupled with high **lytic activity** against **MDR strains**.
- Phage **F2** exhibits high lytic activity and lacks both **virulence factors** and **antimicrobial resistance (AMR) genes**. Following transduction frequency validation, its genomic safety and lytic potency establish it as a prime candidate for future therapeutic cocktails in **phage therapy** and **biological control**.

References

- Jamalludeen, N., Johnson, R. P., Friendship, R., Kropinski, A. M., Lingohr, E. J., & Gyles, C. L. (2007). Isolation and characterization of nine bacteriophages that lyse O149 enterotoxigenic Escherichia coli. *Veterinary Microbiology*, 124(1-2), 47-57. <https://doi.org/10.1016/j.vetmic.2007.03.028>
- Hall, Gina y Ramírez, Alison (2021). Aislamiento y caracterización de bacteriófagos específicos para cepas de Escherichia coli y Salmonella spp. a partir de aguas residuales. <https://up-rid.up.ac.pa/6366/>