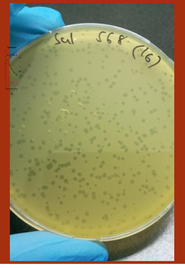
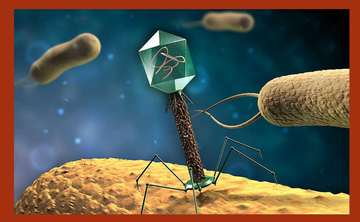


Stability of novel non-typhoidal *Salmonella* phages in simulated gastric fluid and *in vitro* efficacy of silica vesicle to protect phages

Amos L. Mhone[1], Angela Makumi[1], Linda Guantai[1], Josiah Odaba[1], Anna Lacasta[1], Nicholas Svitek[1]

[1]Animal and Human Health Program, International Livestock Research Institute (ILRI), Nairobi, Kenya



INTRODUCTION

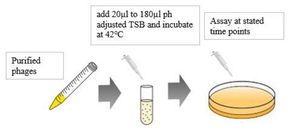
- The zoonotic Multi-Drug Resistant (MDR) non-typhoidal *Salmonella* (NTS) *enterica* serovar Enteritidis is one of the major causes of foodborne infections worldwide.
- Current methods of controlling *Salmonella* infections at the farm level include the use of antibiotics, particularly in poultry farming.
- An estimated 75% of antibiotics administered to poultry are released in the environment and contribute to the emergence of antimicrobial resistance (AMR).
- Bacteriophages are a potential alternative to fight MDR NTS. Phages stable at low pH and high temperatures would render them more suitable for the control of *Salmonella* in poultry as they have a higher chance to survive the harsh gastrointestinal environment

OBJECTIVES

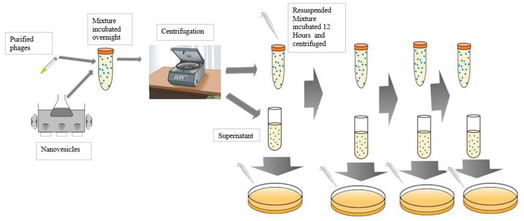
- This study tested the thermal and pH stability of 13 different S. Enteritidis specific phages, previously selected from a cohort of phages based on Restriction Fragment Length Polymorphism (RFLP) patterns.
- Three novel silica vesicles (SV 100, SV 140, and SV 100 C18) were used to test whether they increased the survival of phages in simulated gastric fluid (SGF)

MATERIALS AND METHODS

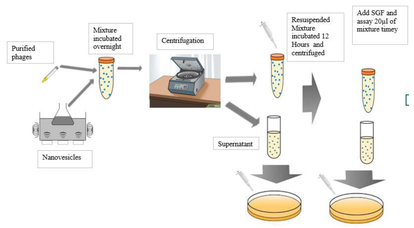
- **Thermal and pH Stability**



- **Rate of phage release from SV**



- **Effect of SV on phage survival**



RESULTS

- All 13 phages were relatedly stable from pH 4 to 12 after 24 hours of incubation with an average titre of 8.1 x10^9 PFU/ml, while they all lost their viability within 3 hours at pH 2-3.
- The thirteen phages were relatively stables from temperatures ranging from 25°C to 42°C after 12 hours of incubation but started losing their viability at 50°C.
- All three Novel Silica Vesicles demonstrated a low but longer rate of phage release upon adsorption for 96 hours.
- Preliminary data indicate that SV 140 C18 nanoparticles shown the ability to protect phages longer, with an average titter of 6.4 x 10^6 PFU/ml at 60 minutes compared to SV 100 (12.6 X 10^4 PFU/ml) and SV 140 (6.3 X10^4 PFU/ml).
- In contrast, free phages in SGF had an average concentration of 3.7 x10^3 PFU/ml after 60 minutes of incubation.

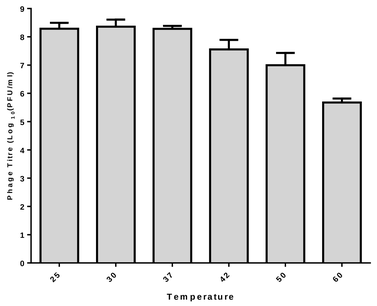


Figure 1: Overall Stability of phages at different temperatures after 12 Hours

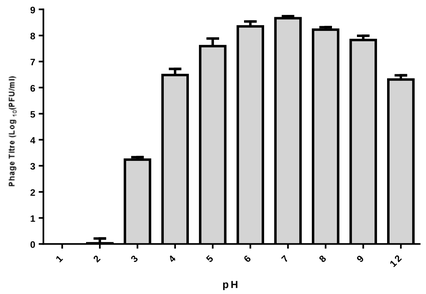


Figure 2: Overall Stability of phages at different pH values after 12 Hours

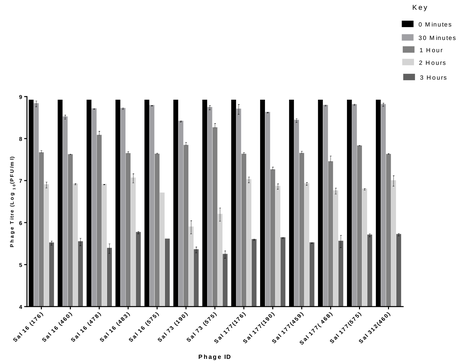


Figure 3: Stability of phages at pH 3 for 3 Hours

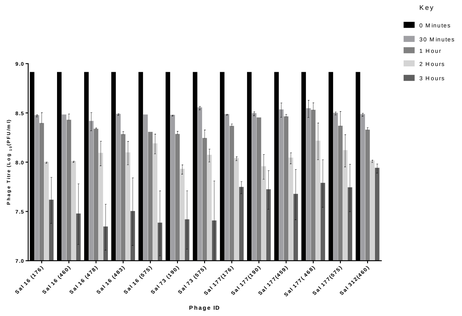


Figure 4: Stability of phages at 42°C for 3 Hours

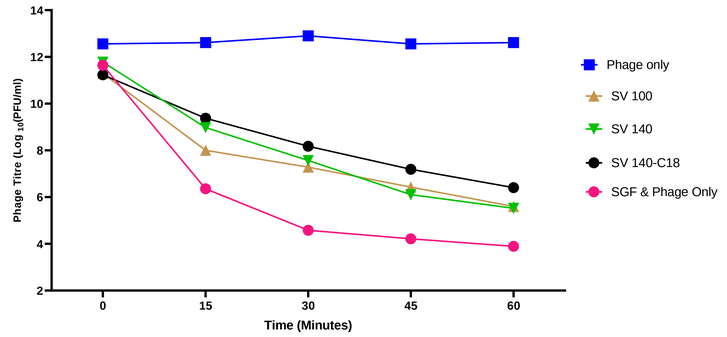


Figure 5: Effect of SV on phage survival

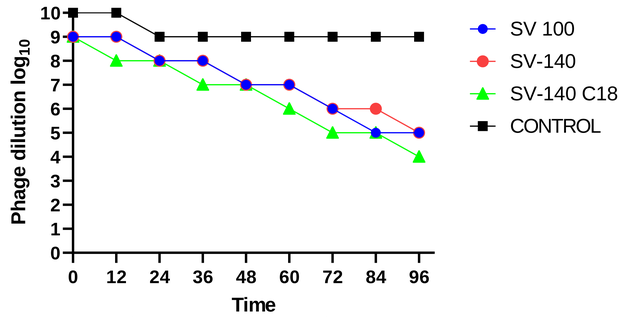


Figure 6: Rate of phage Release from SV

CONCLUSION

- Most phages are relatively stable at 4-9 pH and 25-37°C. Varying phage stability at different pH and temperatures over time indicate varying physiological characteristics of phage
- Phages more stable at low pH and high temperature to be used as potential candidate phages for the in vivo cocktail
- SV demonstrated the ability to protect phages from acidic environment posing as a suitable delivery tool for phages in the gastrointestinal tract

FUTURE WORK

- Evaluate the stability of these phages in vivo in chickens with and without SV.
- Effect of different pH and temperature values on phage-bacterial lysis
- The effect of SGF and SIF on phage-bacterial lysis

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