CHAPTER 1

INTRODUCTION

1.1 Background and significance of the study

Bacteriophages, or phages, are the most abundant biological entities on earth and represent a substantial reservoir of genetic and ecological diversity (Hassan, ElNaby, Elela, Abouelwafa, Sersy, and Fisheries, 2020). Their history spans over a century, starting with their discovery in 1915 by Frederick Twort and independently by Félix d'Hérelle in 1917. Initially studied for their potential as antibacterial agents, phages were used in phage therapy, particularly in the former Soviet Union, before the widespread use of antibiotics (Ofir and Sorek, 2018; Letarov, 2020). Phages act as natural antagonists of bacteria, demonstrating high host specificity. Upon recognizing a suitable host, their receptor-binding proteins mediate the injection of phage genetic material into the bacterial cell. Lytic, or virulent, phages then replicate their genome, assemble new phage particles, and ultimately lyse the host cell to release progeny virions (Gutiérrez and Calap, 2020; Kasman and Porter, 2022). In the early 20th century, initial attempts to employ bacteriophages for therapeutic purposes were constrained by limited understanding of phage–host interaction mechanisms. As antibiotic therapies become increasingly effective and widely adopted, scientific interest in the bactericidal potential of phages gradually declines (Połaska and Sokołowska, 2019). The growing concern over antibiotic resistance has sparked renewed interest in phage therapy as a potential solution. However, phage therapy had limitations due to the sensitivity of phages to stressful environmental factors. Phages were destroyed at high temperatures and low pH. The oral administration of phages targeting core zoonotic bacteria in the animal digestive tract is challenging due to the difficulty in controlling the survival rate of phages because of the loss of viability throughout the digestive tract (Ranveer et al., 2024).

Salmonella enterica subsp. enterica remains a major public health concern, particularly in developing countries, where it continues to be a leading cause of foodborne illness. (Eng, Pusparajah, Mutalib, Ser, Chan and Lee, 2015). This bacterial

subspecies is classified into numerous serovars based on variations in surface antigens, which contribute to its widespread prevalence and epidemiological diversity (Relaño et al., 2021). As a foodborne pathogen, *S. enterica* causes infectious gastroenteritis worldwide and is primarily transmitted through the consumption of contaminated animal-derived products, especially poultry products such as raw chicken and eggs. Upon ingestion, the bacteria survive the acidic gastric environment and colonize the small intestine, leading to gastrointestinal symptoms that range from mild discomfort to severe illness (Vargas, Sánchez, Hernández and Barragán, 2020; Dougan and Baker, 2014). Patients commonly present with clinical symptoms such as nausea, vomiting, diarrhea occasionally hemorrhagic and fever (Fàbrega and Vila, 2013). The World Health Organization (WHO) reports that *Salmonella* infections affect an estimated 550 million individuals annually, including 220 million children under five years of age (Makalatia et al., 2021). The rising incidence of *S. enterica* infections constitutes a critical public health concern globally, with substantial implications for food safety, especially in poultry products, which represent a key source of dietary protein worldwide (House, Bishop, Parry, Dougan and Wain, 2001). The primary treatment for *Salmonella* infections relies on antimicrobial therapy (Lamichhane et al., 2024). However, inappropriate and excessive use of antibiotics in human and veterinary medicine contributes significantly to the escalating problem of antimicrobial resistance (Ahmed, Hussein, Qurbani, Ibrahim, Fareeq and Mahmood, 2024). Forecasts suggest that by 2050, antibiotic-resistant pathogens may account for approximately 1.91 million deaths annually, with an additional 8.22 million fatalities attributed to complications associated with antimicrobial resistance (Naddaf, 2024).

Consequently, the World Health Organization (WHO) underscores the urgent need to develop innovative strategies to mitigate this escalating antimicrobial resistance crisis (Vargas et al., 2020). Biocontrol serves as an alternative strategy to reduce reliance on antibiotics, with bacteriophages (phages) emerging as a promising therapeutic modality (Kimminau et al., 2020). On September 21, 2016, the United Nations General Assembly convenes to address the pressing issue of antibiotic resistance, acknowledging it as one of the most significant global challenges and threats. Among the key recommendations is the reintroduction of phage therapy, which presents several advantages over antibiotics, including host specificity, self-

amplification, biofilm degradation, and low toxicity to humans (Lin, Koskella and Lin, 2017).

Numerous studies explore the potential of phage therapy in mitigating *Salmonella* contamination. For instance, Kimminau et al. (2020) investigated the application of bacteriophages at a concentration of 10^8 PFU/g in chicken feed (1 kg/ton and 1.5 kg/ton of phages) contaminated with *Salmonella enteritidis*. The feed was administered to chicks, and after one-week, fecal samples and organ tissues (liver and spleen) were collected to assess bacterial reduction. The results demonstrated a significant reduction in *Salmonella enteritidis* prevalence in feces ($P<0.001$), underscoring the efficacy of phages in mitigating pathogen contamination in chicken feed during passage through the digestive system, even in the absence of antibiotics. Similarly, Colom et al., (2017) encapsulated bacteriophages using alginate/$CaCO_3$ and evaluated their stability in comparison to free phages in simulated gastric fluid at pH 2.8. The results revealed that free phages exhibited a substantial reduction of 5–8 log PFU/mL after 60 minutes, whereas encapsulated phages showed a comparatively smaller reduction of 3–3.5 log PFU/g. Although encapsulation enhanced phage survival, a notable loss still occurred within the first hour of incubation. These findings underscore that, although oral administration of phage therapy is feasible, the low pH of the stomach, along with the enzymatic activity of bile and the small intestine, remains a critical barrier to its effectiveness (Colom et al., 2017). Consequently, ongoing research into protective strategies and materials is essential to enhance phage stability in harsh gastrointestinal environments, thereby enabling the optimal utilization of phage therapy.

Over the past decade, extensive research was conducted on cell encapsulation within porous materials, demonstrating its effectiveness in minimizing cell loss and enhancing the stability of biotherapeutic agents. Various biopolymers including polysaccharides, synthetic polymers, and proteins were widely employed as encapsulating materials due to their capacity to provide protective barriers against environmental stressors. Among these, the most commonly utilized encapsulation systems involved the formation of small polymeric beads ranging from 1 to 5 mm in diameter. Notably, encapsulation using natural polymers received considerable attention, as it offered a gentle, biocompatible approach that preserved the viability of living cells (Willaert and Baron, 1996). One of the most promising applications of

encapsulation technology is phage immobilization, which prevents the leakage of encapsulated bacteriophages while enabling their controlled release at the target site. Alginate, a natural polysaccharide, is widely recognized as a preferred polymer for phage encapsulation due to its acid resistance and its ability to support the sustained release of viable microorganisms such as probiotics, bacteria, and phages into the gastrointestinal tract (Zhou, Xu, Yu, Han, Liu and Qu, 2022). Several studies investigate the potential of encapsulation to enhance the survival of beneficial microorganisms under harsh digestive conditions. Zhang, Zheng, Gu, Ni, Wang and Tang, (2015) investigated the encapsulation of the probiotic strain *Enterococcus faecalis* HZNU P2 utilizing a composite matrix of alginate and soy protein isolate. Their study demonstrated that the encapsulated probiotics retained high viability following two hours of incubation in simulated gastric fluid (pH 2), whereas the unencapsulated counterparts exhibited a viability reduction of approximately 2 log CFU/mL. Furthermore, upon exposured to a bile salt solution (pH 2.5) for the same duration, encapsulated probiotics exhibited only a minimal decline in viability, while unencapsulated cells failed to survive. These findings suggested that the incorporation of soy protein isolate into the alginate matrix significantly enhanced the resilience of probiotics under harsh gastrointestinal conditions. Despite these promising outcomes, the use of plant-based proteins as encapsulation materials remains relatively limited. In a related study, Vazquez, Calleros, Buendia, Chavez, Ramirez and Carter, (2015) investigated the potential application of cassava starch–modified sodium alginate for the encapsulation of chlorogenic acid (CGA) and assessed its release behavior under simulated gastrointestinal conditions (pH 2.3 and bile salt solution) over a two-hour period. Their findings indicated that the optimal formulation, incorporating 0.75% cassava starch, significantly minimized CGA leakage compared to sodium alginate alone. These results highlighted the promising role of cassava starch and other plant-derived biopolymers in enhancing the protective efficacy of alginate-based encapsulation systems. Overall, these studies highlight the feasibility of utilizing alginate-based encapsulation, supplemented with soy protein isolate or cassava starch, as an effective strategy to improve the stability and controlled release of therapeutic agents, including bacteriophages and probiotics, within the gastrointestinal tract. Ongoing research is needed to optimize

encapsulation formulations and evaluate their practical applications in food safety and biomedical interventions.

The aim of this study is to evaluate the efficacy of calcium alginate beads, modified with varying concentrations of soy protein isolate and cassava starch, in protecting salmonella bacteriophage under different pH conditions, temperatures, and simulated chicken digestive environments, as well as their ability to release viable phages in the target small intestine.

1.2 Research objectives

1.2.1 To study the entrapment efficacy of phages in Ca-alginate beads and Ca-alginate beads with soy protein isolate (SPI) or cassava starch (CS).

1.2.2 To study the stability of salmonella bacteriophage in modified beads under different temperatures and pH levels.

1.2.3 To study the stability of salmonella bacteriophage in modified beads under simulated gastric fluid and bile salt solution.

1.2.4 To study the release of salmonella bacteriophage from modified beads under simulated intestinal fluid.

1.2.5 To study the stability of salmonella bacteriophage in modified beads for six months at 4°C.

1.3 Research hypothesis

The Ca-alginate beads modified with soy protein isolate (SPI) or cassava starch (CS) have a higher entrapment efficiency of *SalP-p*YM phage compared to pure Ca-alginate beads. The stability of *SalP-p*YM phage entrapped in modified Ca-alginate beads under various temperatures and pHs is higher than that of pure Ca-alginate beads. The stability of *SalP-p*YM phage entrapped in modified calcium alginate beads is higher than pure Ca-alginate beads when evaluated under simulated gastrointestinal conditions. Modified calcium alginate beads are more effective in preserving the long-term stability of *SalP-p*YM phage during storage at 4°C for six months compared to pure Ca-alginate beads. The Ca-alginate beads modified with SPI or CS could be applied by mixing with chicken feed to reduce *S.* Typhimurium contamination.

1.4 Expected results

The study's expected result is that Ca-alginate beads modified with soy protein isolate (SPI) or cassava starch (CS) can enhance the entrapment efficiency and increase the survival number of salmonella phages under high temperature and low pH. The survival of phages in the gastrointestinal tract was increased and their release in the intestine was accelerated.

1.5 References

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