Microencapsulation of bio-control bacteriophage by spray drying method

Islam G. Wang Qi*. Sabour P*. Warriner Keith
University of Guelph, Guelph, Canada. Guelph Food Research Center, Agriculture and Agri-Food Canada, Guelph, Canada (gislam@uoguelph.ca)

INTRODUCTION AND OBJECTIVE

Lytic bacteriophages are host specific and multiply by lysing (killing) the host cell.

In recent years, use of bacteriophages as bio-control agents of pathogens including *Salmonella*, *Campylobacter*, *Escherichia coli* and *Listeria* especially in the food industry and animal production has been recognized in the USA and Europe. Due to emerging antibiotic resistant strains among the pathogens, therapeutic application of bacteriophages has been suggested as a possible alternative to antibiotics.

For effective treatment of intestinal pathogens it is imperative that bacteriophages survive the acidic environment of the stomach and reach the digestive tract.

Encapsulation can protect phages from the hostile gut condition; can help deliver phages to the digestive system through direct feeding and control their release in the gut when appropriate formulation strategy is implemented. Although spray drying technology is well established and readily adopted for large scale production, the high temperature and pressure involved in the process present a big challenge for it to be used in encapsulation of phage.

The present study was aimed at 1) to efficiently encapsulate bacteriophages by spray drying method. 2) to test the survival of microencapsulated bacteriophages after spray drying and in simulated gastrointestinal condition of pig. 3) to test the release property of encapsulated phage in simulated intestinal fluid (SIF) at pHs 6.8 and 7.2.

MATERIALS AND METHOD

Bacteriophage Lysate.
Felix O1 was selected as lytic bacteriophage model due to its broad host range among *Salmonella* species. Felix O1 was propagated in liquid lysate using *Salomonella Typhimurium* DT104 NaI^R host and purified using caesium chloride density gradient method.

Encapsulation by Spray Drying.
Microencapsulation of Felix O1 (3ml x 10^8 pfu/ml) was carried out using a Yamato spray dryer ADL 310 (Yamato Scientific America, Inc, CA, USA) at inlet temperature of 70-90°C. Loading solution for the spray drying process as shown in Figure 1 were prepared by adding purified Felix O1 lysate with the excipients i.e. skim milk powder (SMP), maltose (Mal), polysorbate surfactant at 1:1:1 ratio and emulsified at medium speed. The solution was then added to enteric polymer solution (Eudragit S100, Evonik industries, NJ, USA).

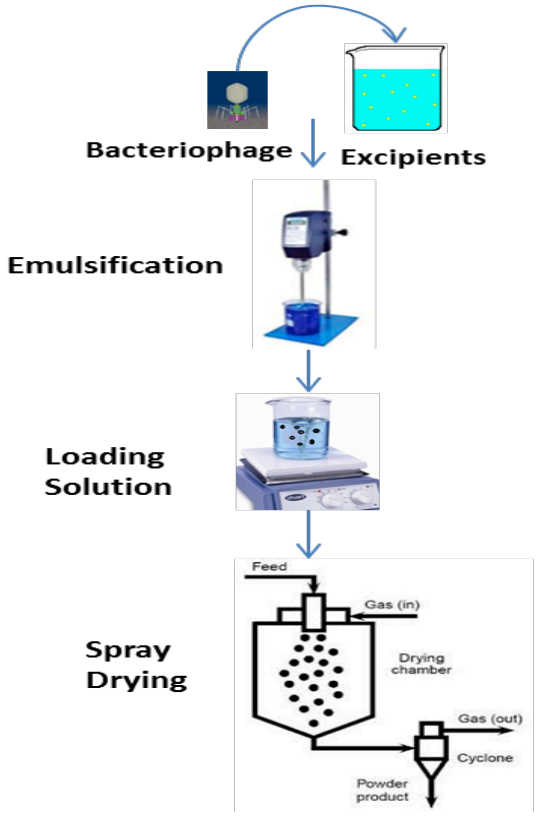

Figure 1: Spray drying process

Microscopy and particle size.
Images of microencapsulated Felix O1 spray dried particles were captured, examined and particle size was measured under a light microscope with calibrated objectives.

Survival of encapsulated phage in Simulated Gastric Fluid (SGF).
A total of 100 mg spray dried microparticles was added to each of 10 ml of pre-warmed (37°C) SGF at pH 2.0 and 2.4 and incubated at 37°C for 0, 5, 15, 30, 60, 90, and 120 min The incubation was terminated by placing the microspheres in 10 ml of SM buffer (pH 7.5) and plaque assay carried out.

Release of bacteriophage from spray dried particles.
Spray dried microparticles (100 mg) were added to

10 ml of pre-warmed SIF at pH 6.8 and pH 7.2 and incubated at 37°C with shaking at 100 rpm at 0, 1, 2, 3, 4, 5, 6 hours. 100 µl of this solution was taken out at time points for phage plaque assay and the same volume of fresh medium was added to replace the volume of the withdrawn samples.

Statistical Analysis.
Performed using Graphpad Prism 5.0 software.

RESULTS AND DISCUSSION

Microscopic analysis (Figure 2) revealed spray dried powder particle of varying sizes ranging 200 nm to 1 µm from 73.04% particle yield. Encapsulation efficiency of 46.15% was achieved.

a) b)

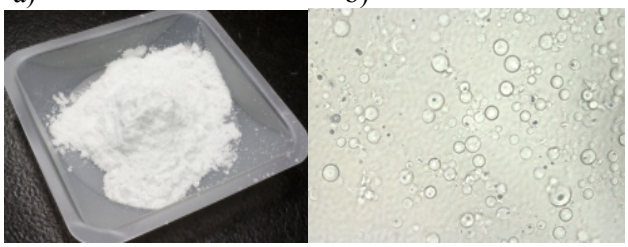

Figure 2: a) Microencapsulated Felix O1 spray dried powder a) bulk b) microscopic image.

In order to determine the protection of the bacteriophages while passing through the hostile stomach condition the Felix O1 loaded particles were treated in SGF (Figure 3). The phage concentration though reduced by 1 log pfu/ml, significant level of resistance towards the acidic pH was displayed by the spray dried powder. Not much variation observed between SGF pH 2.0 and 2.4 in terms of phage titre.

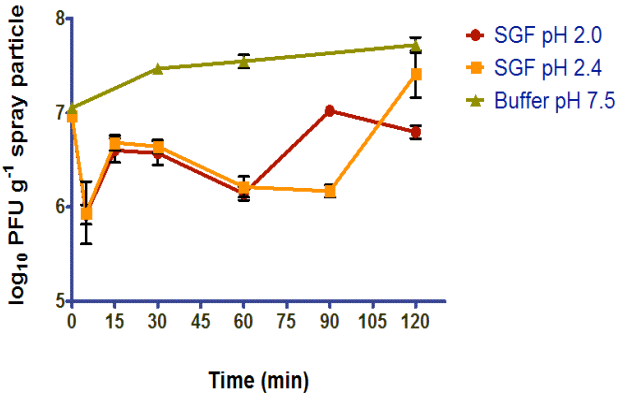

Figure 3 : Survival of bacteriophage after treatment with SGF prepared using 3.2 mg/ml pepsin in 0.2% (wt/vol) NaCl at pH 2.0 and 2.4. SM buffer pH 7.5 used as control.

When the microencapsulated phage particles were subjected to SIF (Figure 4) at pH 6.8 and 7.5, the phage release occurred instantaneously within 1 hour. This could be because of the smaller particle size and the nature of Eudragit polymer which dissolves above pH 6.8.
No significant difference in phage release was observed in SIF of pH 6.8 and 7.2.

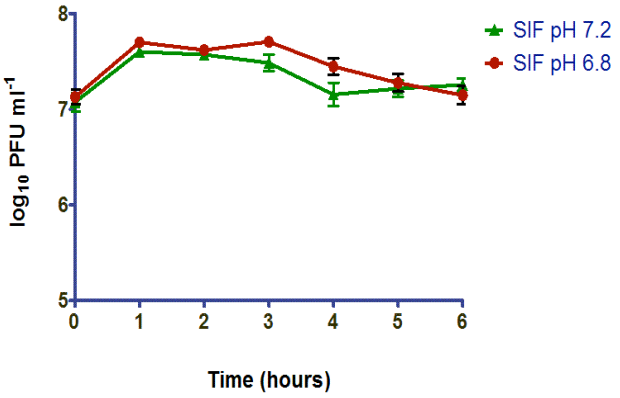

Figure 4 : Release of microencapsulated phage Felix O1 in SIF prepared using 10 mg/ml pancreatin in 50 mM KH2PO4 at pH 6.8 and pH 7.2.

CONCLUSIONS

Bacteriophage indented for phage therapy can be encapsulated by spray drying method.

The spray drying formulation used in this study allows the bacteriophages to survive high inlet temperature and post spray drying conditions.

The spray dried particles can protect the encapsulated bacteriophages from hostile acidic condition with the help of enteric polymer present in the formulation.

Microencapsulated spray dried particles can release Felix O1 phage at pH above 6.8.

REFERENCES

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