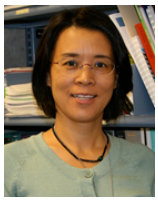
Application of microencapsulation in the development of alternatives to antibiotics for food animal productions



Wang Q., Ma, Y., Zhang, Y., Tang, Z., Gong, J., Sabour, P., Chambers, J., de Lange, C.
Guelph Food Research Center, Agriculture and Agri-Food Canada, Guelph, Ontario, Canada

INTRODUCTION AND OBJECTIVE

Widespread use of antibiotics for growth promotion and disease control in food animal productions has contributed to an increase in antibiotic resistance in pathogenic bacteria. These bacteria can spread from animals to cause infections in humans. There is an increased interest in seeking alternatives such as bacteriophage (phage) to conventional antibiotics. Phages are viruses that infect and kill specific host bacteria, thus do not infect animal and plant cells. This makes them a potentially safe alternative to antibiotics. As well, many essential oils extracted from plants show potent antimicrobial properties when measured under lab conditions. However, uses of phage and essential oils for the control of pathogens in animals have limited success so far. One of the challenges is to deliver sufficient phage or essential oils into the specific intestinal regions where pathogens are harboured. Many phages are sensitive to physical and chemical conditions such as heat, gastric acid and digestive enzymes. Essential oils usually are rapidly absorbed in the upper gastrointestinal tract (GI) after oral administration and, thus are not able to reach the intestine at a sufficient amount. The objective of this research was to study the use of microencapsulation for effective delivery and controlled release of phage and essential oils.

MATERIALS AND METHODS

Microencapsulation of bacteriophage
Phage Felix O1 was obtained from Felix d'Hérelle Reference Center (Université Laval, Quebec, Canada). *Salmonella* Typhimurium DT104 (ATCC 700408) was used for propagating and enumerating phage Felix O1.

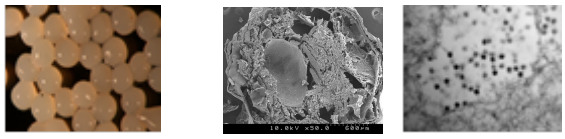
Whey protein (Davisco Foods Intl., USA), an ultra-low viscosity sodium alginate (LBA) (Manugel, FMC International, Ireland) and a low viscosity sodium alginate (Sigma, USA) were used at different ratios for encapsulating phage. Microspheres were made using an Inotech Encapsulator IER-50 (Inotech Biosystems Intl. Inc.) by extruding the phage loaded polymer mixture into $CaCl_2$ solution, followed by further coating with chitosan (Sigma, USA., de-acetylation >85%).

Microencapsulation of essential oils
A sample of essential oil, carvacrol, was encapsulated by the emulsification-extrusion approach using the alginate based formulation as described in the phage section. Another sample essential oil, trans-cinnamaldehyde (CIN), was encapsulated by a melt-solidification approach. Liquid CIN was adsorbed onto magnesium aluminum silicate powder (Fuji Health Sciences, NJ) together with a melted fatty acid and then transformed into solid granules by lowering the temperature in an aqueous suspension An enteric coating of Eudragit L100 was applied using a Glatt fluidized bed spray coater.

In vitro and in vivo characterization of microspheres
The morphology of capsules was observed by light microscopy, SEM, and TEM. . The release profiles of encapsulated phage or essential oils were first tested in simulated gastric and intestinal fluids (SGF and SIF). Selected products were then tested in animal models of chicks and piglets.

RESULTS AND DISCUSSION

Microencapsulation of bacteriophage
Phage loaded microspheres were successfully prepared from alginate-whey protein formulations with a high phage content of 10.6 – 10.8 $\log_{10}$ PFU g^{-1} microspheres. The encapsulation efficiency of viable phage was in the range of 20%-99% depending on the formulation. This indicates that phage was efficiently entrapped in the alginate-whey protein gel matrix during the encapsulation process.



(a) (b) (c)

Figure 1 : Micrographs of alginate - whey protein microspheres loaded with phage Felix O1 by optical microscopy (a), TEM (b), and SEM (c).

Free phage Felix O1 was completely inactivated in SGF at the pH lower than 2.5 following 1 min of incubation. When it is encapsulated in alginate-whey protein, or alginate-only microspheres, the stability of phage in SGF was dramaticly improved. The alginate-whey microspheres provided better protection to Felix O1 than the chitosan coated alginate microspheres reported earlier (Ma et al., 2008). A wide spectrum of release profiles was obtained in simulated intestinal fluid by varying the polymer ratios and total polymer concentration as shown in Figure 2.

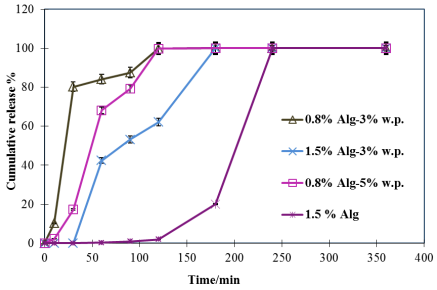


Figure 2 : Releases profiles of phage from alginate/whey protein microspheres in SIF.

When tested in young chicks, it was found that phage encapsulated in alginate only microspheres could not be released sufficiently within the gastrointestinal tract. Addition of whey to the alginate microsphere accelerated the release rate, leading to most phage being released within the GI tract.

Microencapsulation of essential oil

Alginate-whey protein microspheres made by the emulsification-extrusion method were also a good carrier for hydrophobic essential oils such as carvacrol. The encapsulation efficiency was over 95% with the original emulsion oil content of 20%. The dried microspheres contained ~ 70% carvacrol. All the microspheres remained intact after 2 h incubation in SGF, but a small amount of carvacrol, ranging from 10~18% depending on the formulation, was released. The encapsulated carvacrol was completely released in SIF within 2~3 hs (Fig. 3). The release rate could be controlled by varying the polymer compositions. An encapsulated carvacrol having a medium release rate was selected and tested in piglets. The free and encapsulated carvacrol were mixed with the feed and fed the pigs. It was found that the amount of carvacrol absorbed at the upper GI tract was markedly reduced by the encapsulation (Table 1). Further research is undertaken to improve the encapsulation formula for maximizing the delivery of carvacrol to the lower intestinal region.

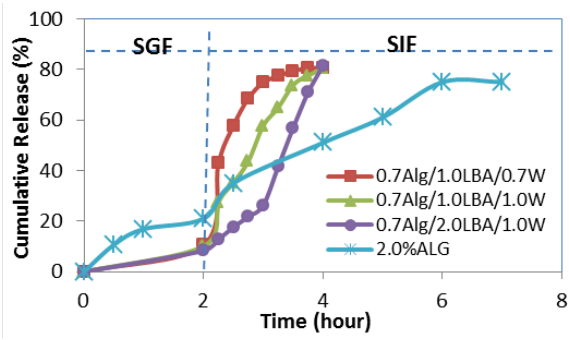


Figure 3 : Examples of release profiles of carvacrol from alginate/whey microspheres in SGF and SIF.

Table 1 The cumulative absorption of free or encapsulated carvacrol in different GI sections of pigs

Intestine Section	Free Carvacrol (%)	Encapsulated Carvacrol (%)
Stomach	58±8	23±13
Duodenum	66±21	29±9
Jejunum	99±1	44±14
Ileum	95±1	82±11
Cecum	100±1	97±1
Colon	99±2	98±2

The CIN microspheres made by the melt-solidification approach provided another way of delivering essential oils. Without the enteric coating, more than 80% of CIN was released from the granules within 2h in SGF and SIF. The enteric coating greatly reduced the loss of CIN in SGF and allowed a selective release in SIF.

When tested in young chicks by oral gavage, it was found that such encapsulation significantly increased the CIN concentration in the jejunum and ileum (Fig 4); however, formulation improvement is still required to further increase the amount delivered to the lower intestines. In particular, the mechanical strength of the granules needs to be enhanced.

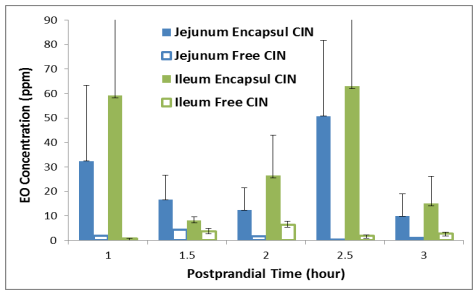


Figure 4 : CIN concentration in jejunum and ileum of young broilers after oral gavage of free or encapsulated CIN.

CONCLUSIONS

The current study indicates that microencapsulation is a potential tool to increase the amount of antimicrobial agents to be delivered to the lower intestine of animals to enhance their efficacy against food-borne pathogens. It is critical to verify each encapsulation formula by animal trials.

ACKNOWLOGIMENT

We are thankful to Yu H., Huang, X., Baxi, S., Hernandez, M., Wu Y., Yin, X. at AAFC and Zhu, J. at the University of Guelph for their technical supports.