# Stability and function of an encapsulated probiotic bacterium for foodborne pathogen control

# Introduction

- Lactobacillus are commonly used as probiotics to dampen enteric infection and promote animal gut health. However, they are heat sensitive, which limits their application. We developed a novel spray-drying encapsulation technology that resulted in approximately 0.5-log reduction of Lactobacillus zeae LB1, a probiotic with the function to control Salmonella and enterotoxigenic E. coli in vivo.
- In the current study, encapsulated LB1 was evaluated for its stability during storage and feed pelleting and for the function on pig gut health.

# **Materials and Methods**

#### **Encapsulation of probiotics**

The probiotic culture (L. zeae LB1) was encapsulated using the method described previously (Liu ea al. 2015).

#### Survival of encapsulated LB1 after storage

After 14 month-storage at at 4°C and 22°C, the content of LB1 was measured to test the storage stability of encapsulated LB1.

## Survival of encapsulated LB1 after pelleting

100 g of encapsulated LB1 (1.4×10<sup>9</sup> CFU/g) was added in 49.9 kg of mash feeds and mixed well, the expected final concentration of encapsulated probiotics was 2.8×10<sup>9</sup> CFU/kg feed. Meanwhile, 300 ml of fresh LB1 culture (5×10<sup>8</sup> CFU/ml) was mixed with 50 kg of mash feed to get the final concentration of LB1 at  $3 \times 10^9$  CFU/kg feed, which serves as a control. 6 mash feed samples with 500 g of each before pelleting were collected in zip bags and stored in 4°C. 6 pelleted feed samples with 500g of each were collected at different time points (4 samples before cooling and 2 samples after cooling) during the pelleting (temperature setting: 80°C; speed: 25 kg /min) and stored in zip bags at 4°C. The fresh LB1 control feeds were also treated as described above. All the feed samples were analyzed for probiotics contents after being stored at room temperature for 7 and 30 days, respectively.

## Animal trial design and sample collection

80 newly-weaned piglets were equally allocated to five groups: 1) basal diets (control, CTL); 2) basal diets supplemented with non-encapsulated LB1 (1x108 CFU/pig per day, NEP); 3) basal diets supplemented with encapsulated LB1 (1x108 CFU/pig per day, EP); 4) basal diets supplemented with 5% bovine colostrum (BC); 5) basal diets supplemented with EP and BC (EP-BC, same dose as in Group 3 or 4).

After five days' treatment, the gastrointestinal tract of euthanized piglets were excised for tissue and intestinal samplings. Intestinal content from the ileum, caecum and colon were also collected for analysis of microbial populations.

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Results				
he storage test, the concentration of encapsulated LB1 was	Table 1. Probiotic contents after pelleting			
ally 1.17×10 <sup>5</sup> CFU/g powder. After 14-month storage at 4°C $22 ^{\circ}$ C, the concentration was decreased to $9.7 \times 10^8$ and $3 \times 10^8$ CFU/g powder, representing 17.1% and 85.6%	Feed samples	Probiotic contents after 7 days storage (CFU/g)	Probiotic contents after 30 days storage (CFU/g)	
iction, respectively.	Mash non-encapsulated	$1.6  imes 10^{6}$	$5.0  imes 10^{4}$	
piotic contents of all the feed samples in the feed pelleting test showed in <b>Table 1</b> . The initial concentration of LB1 was	Pellet non-encapsulated	$2.5  imes 10^{4}$	$2.0  imes 10^4$	
× 10 <sup>6</sup> CFU/g for the encapsulated form and $3.0 \times 10^6$ CFU/g	Mash encapsulated	$2.3  imes 10^{6}$	$1.6  imes 10^{6}$	
the non-encapsulated form.	Pellet encapsulated	$4.0 \times 10^{5}$	$2.8 \times 10^{5}$	

• For the pig trial test, the treatment groups showed no significant difference to the CTL group in growth performance. Supplementation of LB1 or colostrum individually did not affect the population size of Lactobacillus in the ileum and colon of pigs. However, the EP-BC group had a significantly increased population of Lactobacillus in both the ileum and colon (94.57-fold and 23.51-fold, respectively) compared with the CTL group (Figure 1).



Figure 1. Fold changes of *Lactobacillus* population in ileal and colonic digesta from pigs with different treatments.

All values were caculated using the method of  $2^{-\Delta\Delta Ct}$  (value >1.0 represents up-regulation; value < 1.0 represents down-regulation) and expressed as mean $\pm$ standard deviation. Different letters represent significantly different (P < 0.05) from each other.

Conclusion

• Spray-drying encapsulation is an effective method to protect *Lactobacillus* storage and feed pelleting.

• Supplementation of both LB1 and colostrum promoted *Lactobacillus* 

ileum and colon of newly-weaned piglets.



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