**Table S1:** Characteristics of the *Escherichia coli* strain 8185 used in the present study.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameter | Characteristic | | | |
| Origin: | Pig feces |  |  |  | |
| Hemolysis: | beta Hemolytic |  |  |  | |
| Toxin: | Heat-labile toxin [detected with VET-RPLA (Denka Seiken, Tokyo, Japan)]. | | | |
| Adhesion factor: | F4 [detected with Toxigenic Escherichia coli Pili Antisera "SEIKEN" Set (Denka Seiken)]. | | | |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Gene-based characteristics | |  |  |  |  |  | |
| Toxin genes (Product) | Detection | Reference |  | Adhesion genes (Product) | Detection | Reference |
| *stx1* (Stx1) | Negative | [18] |  | *faeG* (F4) | Positive | [19] |
| *stx2* (Stx2) | Negative | [18] |  | *famC* (F5) | Negative | [19] |
| *estA* (STa) | Negative | [18] |  | *fasA* (F6) | Negative | [19] |
| *estB* (STb) | Positive | [19] |  | *fedA* (F18) | Negative | [19] |
| *elt* (LT) | Positive | [12] |  | *fimF41a* (F41) | Negative | [19] |
| *ast1* (EAST1) | Positive | [19] |  | *eaeA* (intimin) | Negative | [18] |
|  |  |  |  | *paa* (pAA) | Negative | [19] |
|  |  |  |  | *aah* (AIDA1) | Negative | [19] |

This strain was stocked in the Dorset Egg medium (Nissui, Tokyo, Japan) and regrown by TS broth. Regrown bacteria were used for the infection study.

After 4 hours of incubation with gentle shaking in a 500 mL Erlenmeyer flask, using TS broth (200 mL) as medium, approximately 1×109 CFU/mL of *E. coli* strain 8185 was harvested.

After centrifuging the culture, the cell slurry was harvested and used to fill the chitosan-coated capsules used to deliver the bacteria.

The number of bacterial cells administered to piglets were determined from the remains of the cell slurry, which was cultured on Desoxycholate Hydrogen sulfide Lactose (DHL) agar (Nissui, Tokyo, Japan).

The gene-based characteristics of *E. coli* strain 8185 were obtained by a previously described method [12,18,19].

**Table S2:** Diarrhea defecation ratio and growth performance of piglets infected with heat-labile toxin-positive enterotoxigenic *Escherichia coli* (1010 colony-forming unit/head) in the repeatability validation study.

|  |  |  |  |
| --- | --- | --- | --- |
| Parameters | Days post infection | Non-infection control (C3) | ETEC infection (LT3) |
| Average daily gain | dpi0-3 | 217 (133, 300) | 117 (67, 167) |
| (g/d) | dpi3-9 | 433 (467, 400) | 192 (283, 100) |
| Mean fecal score | dpi1-3 | 0 (0,0) | 0 (0,0) |
|  | dpi4-9 | 0 (0,0) | 0.33 (0.33,0.33) |
| Diarrhea defecation ratio | dpi1-3 | 0 | 0 |
| (%) | dpi4-9 | 0 | 100 |
| Duration of diarrhea (days) | dpi1-9 | 0.0 | 1.0 |
| ETEC numbers in feces | dpi0 | Not detected | Not detected |
| (log CFU/g) | dpi3 | Not detected | 6.33 (6.57, 6.10) |
|  | dpi9 | Not detected | Not detected |

Four piglets were divided into two groups. The experimental design was as the same as that of Experiment 2.

ETEC: enterotoxigenic *Escherichia coli*.

Fecal scores: 0, normal; 1, loose stool; 2, moderate diarrhea; 3, severe diarrhea (scores 2 and 3 were considered "diarrhea").

The mean values of piglet 1 and piglet 2 are shown in the above table.