





Figure S1. Representative inhibitory zones of agar diffusion test showing the antibacterial activity of cleared supernatants prepared from induced (+) and non-induced (-) recombinant probiotic strains. See materials and methods section for the experiment design. The tested pathogens are (A): *Escherichia coli;* (B)*Salmonella;* (C) *Enterococcus faecalis;* (D)*Salmonella enterica;* (E)*Staphylococcus aureus;* (F)*Staphylococcus aureus;* NC: negative control (MRS medium).



Figure S2. Effects of recombinant human lactoferrin cell lysates on the growth of four pathogenic bacterial strains. About 100 ml L. Delbrueckii (host control), L. Delbrueckii/pNZ8148 (vector control), L. Delbrueckii/HLF were induced protein expression for 5 hours using nisin. Cell pellets were harvested, washed by PBS twice, and then disrupted by sonication. Supernatants (cell lysates) were then harvested by centrifugation. Supernatants (200 μ L/assay) was mixed with pathogenic bacterial broth (1 × 10⁴ cfu/mL; 300 μ L) in eppendorf, and these mixers were further incubated for 24 h at 37 °C. Then, 200 μ L of mixtures were further plated onto NA plates to reveal the remaining growth of bacterial colonies. The blank control presented as smear-type bacterial cultures, revealing countless pathogenic bacterial colonies grown on the NA plates. The final concentration of 12.5 μ g/mL chloramphenicol were also used as the control. Arrows indicated the growth of *S. aureus, En. Faecalis,* and *S. enterica.*



Figure S3. Effects of recombinant bovine lactoferrin cell lysates on the growth of four pathogenic bacterial strains. About 100 ml *L. Delbrueckii* (host control), *L. Delbrueckii*/pNZ8148 (vector control), *L. Delbrueckii/BLF* were induced protein expression for 5 hours using nisin. Cell pellets were harvested, washed by PBS twice, and then disrupted by sonication. Supernatants (cell lysates) were then harvested by centrifugation. Supernatants (200 µL/assay) was mixed with pathogenic

bacterial broth $(1 \times 10^4 \text{ cfu/ml}; 300 \,\mu\text{L})$ in eppendorf, and these mixers were further incubated for 24 hours at 37°C. Then, 200 μL of mixtures were further plated onto NA plates to reveal the remaining growth of bacterial colonies. The blank control presented as smear-type bacterial cultures, revealing countless pathogenic bacterial colonies grown on the NA plates. Arrows indicated the grown of individual bacterial colonies, and the induced *L. Delbrueckii*/BLF almost completely blocked the growth of *S. aureus, En. Faecalis,S. enterica,* and *E. coli.*



Figure S4. Effects of recombinant bovine lactoferrin cell lysates on the growth of four pathogenic bacterial strains. About 100 mL *L.Gasseri* (host control), *L. Gasseri*/pNZ8148 (vector control), *L. gasseri*/BLF were induced protein expression for 5 hours using nisin. Cell pellets were harvested, washed by PBS twice, and then disrupted by sonication. Supernatants (cell lysates) were then harvested by centrifugation. Supernatants (200 µL/assay) was mixed with pathogenic bacterial broth (1

× 10^4 cfu/mL; 300 µL) in eppendorf, and these mixers were further incubated for 24 h at 37 °C. Then, 200 µL of mixtures were further plated onto NA plates to reveal the remaining growth of bacterial colonies. The blank control presented as smear-type bacterial cultures, revealing countless pathogenic bacterial colonies grown on the NA plates. Arrows indicated the grown of individual bacterial colonies, and the induced *L. gasseri*/BLF almost completely blocked the growth of *S. aureus*, *En. Faecalis*, *S. enterica*, and *E. coli*.