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## Expression of CRISPR-associated endonuclease in the cecal microbiota of fattening pigs raised without antibiotics

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### Introduction:

Owing to the increased problem of antimicrobial resistance (AMR) in farm animals, the Department of Livestock Development, Thailand, has launched a campaign for antibiotic-free (ABF) animals in livestock production<sup>1</sup>. Fattening ABF pigs did not receive any antibiotics during their lifetime. However, they received oral probiotics containing *Lactobacillus* spp. at the age of 1-3 days to improve growth performance, intestinal morphology, the immune system, and gut health<sup>2</sup>. The controls (CTRL) were fattening pigs raised in the ordinary industrial system. They received prophylactic antibiotics via intramuscular injection or being added to a basic feed for a short period with a pre-slaughter withdrawal time. The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR-associated proteins (Cas) system, which functions in defense against viruses, was found to be widespread in lactic acid bacteria, particularly *Lactobacillus*<sup>3-4</sup>. Hence, the aim of the present study was to investigate the expression of CRISPR-associated endonucleases in the cecal microbiota of ABF pigs, compared to that of CTRL pigs, using a metaproteomic approach.

### Methods:

Cecal samples of ABF (n = 18) and CTRL groups (n = 20) were obtained from a private slaughterhouse located in Chonburi, Thailand. The content and the mucus were randomly collected from 5 positions of each cecum sample using a biopsy punch, mixed with a 1.5 mL RNeasy lysis solution and stored at -80 °C until analysis. Protein samples were extracted and quantified, prior to trypsin digestion. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was analyzed in a linear positive mode with a m/z of 150-2200 using an Ultimate 3000 Nano/Capillary LC System (Thermo Fisher Scientific) coupled to a Hybrid quadrupole Q-ToF impact II (Bruker Daltonics). Protein profiles of CRISPR-associated endonuclease were analyzed using MaxQuant v 2.0.3.0 together with the UniProt database. t-test or Mann-Whitney U Test in R program was used for statistical analysis.

### Results:

In the ABF group, CRISPR-associated endonucleases Cas1 and Cas9 were prominently observed in *Ruminococcus* and *Lactobacillus*, respectively (Fig 1.). In addition, *Lactobacillus* proteins had beneficial effects on gut health with no marked expression of antimicrobial, biofilm and metal resistance proteins.

### Conclusion:

CRISPR-associated endonuclease Cas9, the protective mechanism of bacteria for virus defense, was highly expressed in *Lactobacillus* in the ABF group. ABF pigs may have more beneficial microorganisms in the gut than CTRL pigs.