CONSTRUCTION OF ACK GENE DELETION ENGINEERING BACTERIA OF VEILLONELLA PARVULA AND ITS CHARACTERIZATION

Xing Xin¹, Long Miao¹, Pang Xiao-Yang¹, Wang Zhe², Liu Guo-Wen²

¹Jilin University, ²College of Animal Science and Veterinary Medicine, Jilin University, Changchun, China

Ketosis is one of the most harmful cow diseases, the origin of it is negative energy balance caused by the decrease of dry matter intaked and the insufficiency of propanoic acid. The traditionaly prevental and therapic methods are supplying precursors of glucose such as propionate to the cows. Successful production of propionic acid via ruminal propionic acid-generating bacteria has not yet been reported. To study the cell metabolic network of dominant microbial in rumen and finally improve negative energy balance in periparturient dairy cows, we knocked out acetate kinase gene (ACK) which codes the key enzyme in generation process of acetic acid and constructed the veillonella parvula ack deleted mutant H2Δ(ack). Finally, the fermentation characteristics of the H2Δ(ack) was analyzed.

In this study, we extracted the genome of veillonella parvula, and obtained the two wings of the ACK gene by anchoring PCR. By BLAST, the results showed the sequence obtained was ACK gene. On the basis of the sequence of GenBank that the ACK protein amino acid sequence, we found the active center of veillonella parvula H2 (about 900 bp), introduced a acid-resistant gene between them, and finally it was linked with pUC18 vector building suicide plasmid using homologous recombination successfully. Two engineering bacteria were screened from the plenty transconjugants with selectivity culture medium through donor and receptor bonding test. PCR identification and ACK analysis of enzyme activity revealed that they belong to ACK gene deletion strains resistant fluoroacetic.

Primary substrates were cultured with wild-type strains and genetic engineering bacteria H2Δ(ack) for 48 h respectively in vitro fermentation of ruminal fluid. The results were shown as follows: the lactate in the culture fluid was used by wild-type strain and H2Δ(ack), VFA produced is mainly propionic acid, the type of their fermentation was prone to propionic acid-type. Compared to wild-type strains, the concentration of lactate and the ratio of acetate to propionate were obviously decreased in the culture fluid with H2Δ(ack). The results suggested that the genetic engineering bacteria H2Δ(ack) belonged to a deleted strain of acetic acid-producing gene and it could lay a foundation of developing a new micro ecosystem preparation for prevention and control of the diseases in periparturient dairy cows.

Keywords: Rumen, veillonella parvula, ACK, Gene Deletion, fermentation