The survival of silage lactic acid bacteria in the gut of ruminants assessed by culture-independent microbiota analysis methods
（培養によらない細菌叢解析手法で評価した発酵飼料中乳酸菌の反芻動物消化管における生残性）
buchneri, and Enterococcus faecium are often used as silage inoculants, the LAB species usually detected in the cattle gut are Streptococcus bovis, L. vitulinus, L. ruminis, L. johnsonii, and L. murinus.

In the meantime, the microbial diversity analysis techniques have been developed from culture-dependent to culture-independent methods. Application of the DNA based techniques, especially those based on ribosomal RNA genes, more easily enable to find new species and understand the dynamics of the microbiota in various environmental samples. Using traditional plate-culture, more than 50% of the dominant microbiota are known to be uncultured. Therefore, if we carry out culture-independent microbiota analysis, the LAB communities of silage and the gut content can better be clarified than previously shown by plate-culture. Likewise, because the population of LAB among the total bacteria in the gut is known small, polymerase chain reaction based procedure may help detect LAB in the gut. Accordingly, we performed denaturing gradient gel electrophoresis (DGGE) using Lactobacillus-specific primers.

Three experiments were carried out to assess whether the silage LAB survive in the gastrointestinal tract of ruminants. For the first step, wilted Italian ryegrass silage was prepared in laboratory scale silos, and fed ad libitum to 3 goats equipped with rumen cannulae. Silage was given alone or with concentrates at a 1:1 ratio on a dry matter basis. The LAB communities were compared among silage, rumen fluid, and feces. The LAB detected in the wilted silage included L. plantarum, L. brevis, L. murinus, and L. sakei. Bands indicative of L. murinus were detected in either the rumen fluid or feces whereas the bands indicative of L. plantarum, L. brevis, and L. sakei were not. Sampling times and goat-to-goat variations did not affect the LAB communities found in the rumen fluid. The LAB communities found in the gut were not remarkably affected by the consumption of silage LAB, even when the silage was accompanied by concentrates that facilitate gut fermentation. Therefore, it was concluded that it may be difficult for silage LAB to survive the digestive process in the gut of ruminants.

Secondly, as the follow-up of previous mini-silo and small ruminant study, we performed a practical survey to identify the LAB inhabiting bunker-made whole crop corn silage and the feces of silage-fed dairy cows. To obtain diverse silages produced in practice, we collected 1 representative silage sample and 3 different feces samples from dairy cows on 3 dairy farms in Hua Bei, China and 3 in Kumamoto, Japan. L. acetotolerans was detected in all tested corn silage samples. L. pontis was detected in samples from 2 farms in Hua Bei and 3 farms in Kumamoto, and L. casei was detected in samples from 1 farm each in Hua Bei and Kumamoto. The LAB identified in cow feces included L. acetotolerans, L. pontis, L. casei, Weissella paramesenteroides, and L. diolivorans. Although image analysis of DGGE band patterns indicated clear separation between silage LAB and faecal LAB, 3 of the 8 silage LAB survived digestion and were detected in both silage and feces. These results indicate that, although it may be tough for silage LAB to survive the digestive process, a number of species have the potential to convey their probiotic function from silage to dairy cows.

In the second step survey, farmers used corn silage at a proportion of 0.20–0.40 in the dairy cow diet. Silage LAB were diluted by mixing with other feeds, whereas concentrated feeds are known to acidify the rumen content and thereby increase the competition between LAB and other gut bacteria. Meanwhile, survival of the silage LAB can be scrutinized in greater detail if the ruminal bacterial community is examined together with that of the faeces. If silage LAB are not detected in the feces, it remains undetermined whether their elimination takes place ruminally or post-ruminally.
In Japan, production and feeding of total mixed ration (TMR) silage, which stores entire mass of TMR mixture, has been practiced. Therefore, for the third step, silage, rumen fluid, and fecal samples of dairy cows were collected at 2 different research institutes that offered TMR silage throughout the year. A total of 14 LAB species were found in TMR silage samples, of which 5 (L. acetotolerans, L. pontis, L. casei, L. suebicus, and L. plantarum) were detected in dairy cow feces. Most of the DGGE bands detectable in feces were also seen in the rumen fluid, suggesting that any elimination of silage LAB took place in the rumen rather than in the post-ruminal gut segments.

In this series of experiments, 6 of 21 LAB species found in silage were detected in goat and dairy cow feces. About one-third of the silage LAB was found to have the potential to convey their probiotic function; hence, silage can be regarded as a good vehicle for the propagation and delivery of probiotic LAB. Likewise, among the 6 LAB species assumed to survive in the gut, 4 (L. acetotolerans, L. pontis, L. suebicus, and L. murinus) have never been reported to inhabit silage by plate-culture technique. Therefore, the finding that about one-third of the silage LAB can survive in the gut may stem from the use of culture-independent analysis in this study. Meanwhile, the DGGE procedure does not differentiate among live, injured, and dead microorganisms, which can mislead interpretation of the survival of silage LAB. However, we recently isolated L. acetotolerans and L. pontis as the slow-growing LAB from both TMR silage and feces of the silage-fed dairy cows. Using the isolates and other non-surviving species, further study to examine the reason for the differences in the survival rates of silage LAB can be performed.