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Identification of lactic acid bacteria in the rumen and feces of dairy cows fed with total mixed ration silage to assess the survival of silage bacteria in the gut

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ABSTRACT

The survival of silage lactic acid bacteria (LAB) in the gut of dairy cows was evaluated by examining the LAB communities of silage and gut contents. Samples were collected at 2 different research institutes (Mie and Okayama) that offered total mixed ration (TMR) silage throughout the year. Silage and feces were sampled in August, October, and November at the Mie institute, whereas silage, rumen fluid, and feces were sampled in June and August at the Okayama institute. Denaturing gradient gel electrophoresis using *Lactobacillus*-specific primers was performed to detect LAB species in the samples. The selected bands were purified for species identification and the band patterns were used for principal component analysis. Lactic acid was the predominant fermentation product in all the TMR silages analyzed, and the lactic acid level tended to be constant regardless of the sampling time and region. A total of 14 LAB species were detected in the TMR silage samples, of which 5 (*Lactobacillus acetotolerans*, *Lactobacillus pontis*, *Lactobacillus casei*, *Lactobacillus suebicus*, and *Lactobacillus plantarum*) were detected in the dairy cow feces. Most of the denaturing gradient gel electrophoresis bands for the feces samples were also detected in the rumen fluid, suggesting that any elimination of silage LAB occurred in the rumen and not in the postruminal gut segments. The principal component analysis indicated that the LAB communities in the silage, rumen fluid, and feces were separately grouped; hence, the survival of silage LAB in the cow rumen and lower gut was deemed difficult. It was concluded that, although the gut LAB community is robust and not easily affected by the silage conditions, several LAB species can inhabit both silage and feces, which suggests the potential of using silage as a vehicle for conveying probiotics.

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INTRODUCTION

Lactic acid bacteria (**LAB**) are of primary importance in the process of moist forage crop preservation. Energy losses and protein degradation can be reduced if LAB, particularly homo-fermentative species, predominate the fermentation process (McDonald et al., 1991). Inoculants, such as *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Enterococcus faecium*, and others, are often used to secure desirable lactic acid fermentation (Weinberg and Muck, 1996). After long-term inoculant use, the primary purposes, such as reducing nutrient loss and preventing intensive proteolysis, have almost been achieved. Accordingly, the focus of attention has now shifted to additional functions, such as promoting animal health and disease prevention. As LAB constitutes a part of the ruminant gut bacteria, silage can be considered a vehicle to propagate and deliver probiotic LAB species. Although the original concept regarding probiotics is based on benefits taking place postruminally, certain probiotics may even confer advantages in the rumen, such as improved digestibility and an inhibition of acidosis (McAllister et al., 2011). Animal performance (intake, weight gain, and milk yields) could thus be enhanced with LAB-inoculated silage even when no fermentation improvement is seen; however, the ability for improvement is dependent on the strains used (Weinberg and Muck, 1996).

To ensure that a probiotic-based inoculation is beneficial, the survival of LAB in the ruminant gut needs to be verified. Weinberg et al. (2004) examined the changes in the populations of silage LAB (*L. plantarum, E. faecium*, and *Pediococcus pentosaceus*) during an in vitro rumen incubation and concluded that acceptable numbers of LAB could survive, particularly when sugar substrates were used for fortification (Weinberg et al., 2004). Similarly, Rodriguez-Palacios et al. (2009) isolated *L. plantarum* from the cecum and both *P. pentosaceus* and *Pediococcus acidilactici* from bovine

fecal matter, suggesting that LAB species used as inoculants may survive in both the rumen as well as the intestine. However, as Rodriguez-Palacios et al. (2009) acknowledged, the isolation of *L. plantarum* from the bovine gut is rare. The LAB species usually detected in the gut are *Streptococcus bovis*, *Lactobacillus vitulinus*, *Lactobacillus ruminis*, *Lactobacillus johnsonii*, and *Lactoabacillus murinus* (Krause et al., 2003; Hernandez et al., 2008; Nader-Macías et al., 2008). This raises a debate on how to select LAB species for developing probiotic supplements.

To determine the survival of silage LAB in the bovine gut, we performed a practical survey to monitor the LAB community in bunker-made whole crop corn silage and in the feces of dairy cows receiving the silage (H. Han, C. Wang, Z. Yu, Q. Xu, and N. Nishino, unpublished data). Three (*Lactobacillus acetotolerans*, *Lactobacillus pontis*, and *Lactobacillus casei*) out of 8 silage LAB species were detected in feces, suggesting that although it may be tough for silage LAB to survive the digestive process in the gut, several LAB species may have the potential to act as probiotics when supplemented with silage to dairy cows.

In the previous survey, farmers used corn silage at a proportion of 20 to 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 feces. If silage LAB are not detected in the feces, it remains undetermined whether their elimination takes place ruminally or postruminally. In Japan, production and feeding of TMR silage, a silage that stores the entire mass of TMR mixture, has been practiced. Based on the feeding regimen of TMR silage, gut content samples can be collected from dairy cows that were exclusively fed silage. In our study, the fate of silage LAB was evaluated by examining the LAB community in TMR silage, rumen fluid, and fecal material of dairy cows to see whether the silage LAB are removed ruminally or postruminally.

MATERIALS AND METHODS

TMR Silage, Rumen Fluid, and Fecal Sampling from Dairy Cows

We collected samples of TMR silages produced at the Mie Prefecture Livestock Research Institute (4 bales each on August 23, October 29, and November 2, 2010) and a feed company based in the Okayama prefecture (2 bales each on June 18 and August 20, 2012). The composition of the Mie TMR silage varied depending on production time (Table 1). Three crop (corn, sorghum, and wilted Italian ryegrass) silages were used in the August product, whereas only single crop (wheat) and crop-free silages were used in the October and November products, respectively. For the November product, timothy hay was used instead of crop silage. The CP and total digestible nutrient levels were set at ~15 and ~72% DM by mixing the silage with concentrates and wet by-products. The TMR mixture was then wrapped with 6 layers of plastic film (Shito et al., 2006). For the Okayama TMR products, corn and rice silages were used as ingredients and the recipe was unaltered between the June and August preparations. The nutrient composition was similar to that of the Mie products; however, the Okayama prefecture-derived TMR mixture was stored after vacuum-sealing in a thick (0.1 mm) plastic bag. The mixtures were stored outside for 1 to 2 mo in both regions. Several grab samples were mixed to create a composite sample of ~0.5 kg.

At the Mie Livestock Research Institute, TMR silage was offered ad libitum to dairy cows throughout the year and rectal samples were taken at around 1000 h. As fecal samples collected from 3 cows on 3 consecutive days showed no apparent day-to-day variations in the denaturing gradient gel electrophoresis (**DGGE**) patterns, fecal samples taken on the first day were used for community analysis as representative samples. About 1 g of feces was aliquoted in an Eppendorf tube and shipped frozen to Okayama University. Because some of the cows were dried off between August and November, occasional differences were observed among the dairy cows depending on the collection time.

For the Okayama products, silages were transported from the feed company and stored at the Okayama Prefecture Livestock Research Institute until use. Similar to the Mie Institute, TMR silage was also offered ad libitum to dairy cows throughout the year. We collected the rumen content and rectal samples at around 1300 h from 5 dairy cows. The rumen content was obtained using a flexible stomach tube and then strained through 4 layers of surgical gauze. About 1 g of the rumen fluid and fecal samples were placed in Eppendorf tubes, shipped on ice, and were stored frozen until analysis at Okayama University. Because the 2 sampling times were close, the rumen fluid and feces were taken from the same dairy cows.

Chemical and Bacterial Community Analyses

The silage DM content was determined by oven drying at 60°C for 48 h. The fermented product content in the silage was determined following water extraction