Postnatal changes in the relative abundance of intestinal *Lactobacillus* spp. in newborn calves

Tadashi TAKINO¹,²), Yuko KATO-MORI¹), Daisuke MOTOOKA³), Shota NAKAMURA³), Tetsuya IIDA³) and Katsuro HAGIWARA¹)*

¹) School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai, Ebetsu-shi, Hokkaido 069-0836, Japan
²) Scientific Feed Laboratory Co., Ltd., 3-5 Miyahara, Takasaki, Gunma, Japan
³) Research Institute for Microbial Diseases, Osaka University, 3-1 Yamadaoka, Suita, Osaka, Japan

ABSTRACT. The intestinal microbiota of newborn calves changes during the early postnatal period and influences their health and immune function. We studied the compositional changes in the intestinal microbiome of newborn calves during the first week after birth by metagenomic analysis. In feces from newborn calves, we identified 4 bacterial phyla, namely, Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria. The relative abundance of *Lactobacillaceae* significantly increased from day 1 to day 7. We evaluated *Lactobacillus* spp. colony numbers using selective agar plates and confirmed that the abundance of *Lactobacillus* spp. significantly increased during the first 7 days after birth. In conclusion, *Lactobacillus* spp. colonized the intestinal tract of calves during the first 7 days after birth.

KEY WORDS: bacterial culture, metagenomics analysis, microbiome
Fecal samples were diluted with a buffer that consisted of 0.45 g KH$_2$PO$_4$ (Kishida Chemical, Osaka, Japan), 1.68 g Na$_2$HPO$_4$•12H$_2$O (Kanto Chemical, Tokyo, Japan), 0.05 g l-cysteine HCl•H$_2$O, 0.05 g Tween® 80 and 0.1 g agar in 100 ml of distilled water. Samples were subjected to serial 10-fold (w/v) dilutions with the above-mentioned dilution buffer and vortexed. The Lactobacillus spp. colonies were counted after the samples were cultured on modified Lactobacillus selection (LBS) agar plates (Nissui Pharmaceutical, Tokyo, Japan). The results were expressed as the number of colony-forming units (CFUs) per g of fecal samples. Statistical analysis was performed using SPSS version 11.5 (SPSS Inc., Chicago, IL, U.S.A.). The RAs and colony counts in the microbiota between day 1 and day 7 after birth were calculated, and significance of the differences was analyzed by Student’s t-test.

The sequencing generated a dataset consisting of filtered high-quality classifiable 16S rRNA gene sequences with a mean ± standard error (SE) of 14,317 ± 3,967 sequences per sample.

The metagenomic analysis determined that several phyla were present in the microbiome. The detected bacteria could be subdivided into 4 phyla, namely Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria (Fig. 1). In samples from the first feces on day 1, the RAs (% mean ± SE) of these phyla were 0.2 ± 0.14, 18.1 ± 9.7, 24.0 ± 7.4 and 53.4 ± 17.0, respectively. In fecal samples obtained on day 7, the RAs of Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria were 11.9 ± 2.3, 25.8 ± 3.4, 43.9 ± 4.1 and 13.6 ± 2.2, respectively. These results showed that the intestinal microbiota of newborn calves was composed of 4 phyla: Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria. The RAs of these 4 phyla changed between the 1st and 7th days after birth. All the calves were managed in the same breeding environment and given the same milk replacement diet. Nevertheless, differences in the composition of the intestinal microbiome among individual calves were observed in the day 1 feces. The individual differences in the taxonomic composition of the intestinal microbiota among the calves were smaller at 7 days after birth.

Seven families of bacteria rose to dominance in the microbiota of the tested samples during the first 7 days, as follows: families *Bifidobacteriaceae* and *Coriobacteriaceae* in phylum Actinobacteria; families *Lactobacillaceae*, *Streptococcaceae* and *Veillonellaceae* in phylum Firmicutes; family *Bacteroidaceae* in phylum Bacteroidetes; and family *Enterobacteriaceae* in phylum Proteobacteria (Table 1). *Bifidobacteriaceae* were undetectable, and the RA of *Coriobacteriaceae* was 0.2 ± 0.14 on day 1, while their RAs on day 7 were 2.3 ± 0.29 and 9.3 ± 2.3, respectively. The RAs of *Lactobacillaceae*, *Streptococcaceae* and *Veillonellaceae* changed from 0.14 ± 0.13, 0.16 ± 0.07 and 0.08 ± 0.04 on day 1 to 11.3 ± 5.1, 4.7 ± 2.2 and 7.1 ± 1.6 on day 7. The RA of *Bacteroidaceae* increased from 4.2 ± 2.1 on day 1 to 22.2 ± 4.6 on day 7, and that of *Enterobacteriaceae* decreased
from 51.3 ± 17.2 on day 1 to 10.5 ± 2.8 on day 7, respectively (Table 1). The RAs of the following bacterial families increased significantly from day 1 to day 7: Bifidobacteriaceae ($P<0.001$), Coriobacteriaceae ($P<0.01$) and Veillonellaceae ($P<0.01$), while that of Enterobacteriaceae decreased with borderline significance ($P=0.07$).

One study in adult cattle reveals that Firmicutes dominate the intestinal microbiome, representing 81.9% of all sequence reads, followed by Proteobacteria (9.6%), Bacteroidetes (5.4%) and Actinobacteria (2.8%) [4]. In the present study, the composition of the intestinal microbiome in the first feces varied among newborn calves born under the same environmental conditions. Therefore, some bacteria contained in the mother’s colostrum may contribute to the composition of the intestinal microbiota of newborn calves.

The number of bacterial CFUs on the LBS agar plates increased in all the calves from a mean value of 2.87 log CFU/g on day 1 to 8.76 log CFU/g on day 7 (Fig. 2). Interindividual variability in the detected bacterial species was observed on day 1, but the microbiomes of the calves were more homogeneous on day 7. The number of bacterial CFUs on LBS agar, which is selective for Lactobacillus spp., increased from day 1 to day 7. CFUs in the feces of calves at 7 days old reached the copy number similar to the amount reported for 12-day-old calves [6]. This result is similar to that reported for human infants [4]. The above change corresponds to the results of the metagenomic analysis. In the present study, we detected Lactobacillus spp. in the first feces samples from only 2 of 5 calves, but the population increased to more than $10^7$ CFU/g on day 7. Therefore, the intestinal colonization of newborn calves by Lactobacillus spp. took place during the first 7 days of life.

The intestinal microbiome of newborn calves was found to comprise genetic material from the phyla Actinobacteria,
Bacteroidetes, Firmicutes and Proteobacteria according to metagenomic analysis. The intestinal microbiome was characterized by an increase in the RA of Firmicutes (including families, such as Lactobacillaceae) during the first week after birth. Thus, the first week of life may be an important period for the intestinal colonization of calves by Lactobacillus spp.

ACKNOWLEDGMENTS. This study was supported in part by a Grant-in-Aid for Scientific Research from JSPS KAKENHI (Grant Number 23580427). We thank Mr. Taku Okamura and the staff of Rakuno Gakuen University Farm for their expert technical assistance.

REFERENCES

1. Frank, D. N., St Amand, A. L., Feldman, R. A., Boedeker, E. C., Harpaz, N. and Pace, N. R. 2007. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc. Natl. Acad. Sci. U.S.A. 104: 13780–13785. [Medline] [CrossRef]
2. Lukás, F., Koppová, I., Kudrná, V. and Kopecný, J. 2007. Postnatal development of bacterial population in the gastrointestinal tract of calves. Folia Microbiol. (Praha) 52: 99–104. [Medline] [CrossRef]
3. Meale, S. J., Li, S., Azevedo, P., Derakhshani, H., Plaizier, J. C., Khafipour, E. and Steele, M. A. 2016. Development of Ruminal and Fecal Microbiomes Are Affected by Weaning But Not Weaning Strategy in Dairy Calves. Folia Microbiol. (Praha) 52: 99–104. [Medline] [CrossRef]
4. Mitsuoka, T. 2014. Establishment of intestinal bacteriology. Biosci. Microbiota Food Health 33: 99–116. [Medline] [CrossRef]
5. Rudi, K., Moen, B., Sekelja, M., Frisli, T. and Lee, M. R. 2012. An eight-year investigation of bovine livestock fecal microbiota. Vet. Microbiol. 160: 369–377. [Medline] [CrossRef]
6. Timmerman, H. M., Mulder, L., Everts, H., van Espen, D. C., van der Wal, E., Klaassen, G., Rouwers, S. M., Hartemink, R., Rombouts, F. M. and Beynen, A. C. 2005. Health and growth of veal calves fed milk replacers with or without probiotics. J. Dairy Sci. 88: 2154–2165. [Medline] [CrossRef]
7. Vlková, E., Trojanová, I. and Rada, V. 2006. Distribution of bifidobacteria in the gastrointestinal tract of calves. Folia Microbiol. (Praha) 51: 325–328. [Medline] [CrossRef]