Case Report

Metabolic and Microbial Characterizations for the Gastrointestinal Digesta of the Zoo Colobus guereza

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Introduction

Abyssinian black-and-white colobus (Colobus guereza), one of the major colobine monkeys, inhabits west, central, and east Africa and lives in social groups in the rain forests [1]. A previous study reported that Colobus guereza spends 52-63 % of its time resting and 19-26 % feeding [2] and has an abnormally low basal metabolic rate, which may be linked to their lower activity level [3]. Moreover, they choose valuable food habitats and consume various plant materials, including leaves, seeds, and fruits, depending on their location and the season [4-8]. Colobus guereza consumes mainly leaves and fruits [2]. Therefore, they obtain their dietary energies and nutrients mainly from plant materials, including cellulolic fibers via a foregut-fermentation digestive system similar to that of ruminants [9]. The Colobus monkey has a forestomach consisting of three regions, namely the cardiac gland, proper gastric and pyloric gland regions [10]. The cardiac gland region of the stomach is larger than the other regions and provides the environment for microbial fermentation of plant materials. Actually, microbial fermentation in the foregut of the colobus is similar to rumen fermentation in terms of bacterial counts, digestive enzymes, organic acids, and pH [11-14]. Recently, a metagenome analysis of the feces of the colobus was performed using the next-generation sequencing system [15,16]; however, the whole composition of their digestive microbiome is unclear. Colobus guereza is captured and displayed in zoos, and their care manual [17] provide the appropriate and practical information on the nutrients and feeds for zoo Colobuses based on several basic research [7,18-21]. However, knowledge of the microbial and metabolic features of the digestive tracts of zoo Colobuses is limited. In this study, we obtained digesta samples from the zoo Colobus guereza, which died during anesthesia, and elucidated the general digestive features by analyzing the organic acids, metabolome, and metagenome of the digesta.

Case Presentation

Animal and digesta samples

Abyssinian black-and-white colobus (Colobus guereza, male, 7 years old) was singly reared and housed at the Kamine Zoo (Hitachi-city, Ibaraki, Japan), and died unexpectedly during anesthesia for a regular health examination. After the post-mortem examination by the veterinarian, the complete gastrointestinal tract was removed and the regions were numbered (Nos.1-12) as shown in (Figure 1A); the digesta was collected immediately from each region and stored at -80 ºC until analyses. Before the sampling, Colobus guereza was normally kept in a cage during the daytime and in the next closed bedroom during nighttime. The feed contents for Colobus guereza are shown in (Table S1). Feed was provided three times every day, and water was provided ad libitum. During the daytime, visitors could nearly approach but not directly touch the Colobus guereza in the cage.

Organic acid analysis using ion-exclusion HPLC

Organic acid concentrations in the digesta were measured using ion-exclusion High-Performance Liquid Chromatography (HPLC) as described previously [22]. Organic acids were detected in all the digesta samples, but the digesta from the Pars Pylorica (PP9) had a relatively low concentration of organic acids compared to the digesta from the other regions. Obviously, higher levels of acetate were detected in both the foregut (PS12, TG11, and S10) and the hindgut (C4, PC3, DC2, and R1). Conversely, the concentrations of...
succinate, formate, and \( n \)-valerate were lower than those of acetate and \( n \)-butyrate. Furthermore, lactate concentrations were higher in the digesta from the small intestine (D8, PJ7, DJ6, and I5) than the digesta from other regions (Figure 1B, Table S2).

Microbiota analysis using the miseq platform

Bacterial DNA was extracted from the digesta as described previously [23]. The microbiota composition of the digesta was analyzed using Next-Generation Sequencing (NGS) and the MiSeq platform (Illumina, CA, USA) as previously described [24]. The alpha diversity in each gastrointestinal digesta was different: the hindgut digesta showed higher alpha diversity than the forestomach. Weighted UniFrac principal coordinates analysis showed that the forestomach, small intestine, and large intestine had different microbiota. The top 10 abundant genera in the stomach, small intestine, and large intestine are shown in (Table 1), respectively. As shown in (Tables 2), each gastrointestinal digesta had a unique microbiota. In the forestomach (PS12, TG11, and S10), the genera *Prevotella* and *Selenomonas* were dominant, while the genus *Clostridium* was dominant in PP9 (Table 1). In the small intestine, each digesta (D8, PJ7, DJ6, and I5) also had a unique microbiota, the genus *Turicibacter* was predominant in I5 compared with the other regions (Table 2). In the large intestine, the various digesta (C4, PC3, DC2 and R1) had similar microbiota, relatively the family *Ruminococcaceae* was dominant (Table 3).

Metabolome analysis using gas chromatography (GC)-mass spectrometry (MS)

Metabolome analyses of the digesta in water-soluble low-molecular-weight metabolites were performed using GC-MS as described elsewhere [25]. Although statistical analysis was not performed, palmitic acid levels were relatively higher across all the regions of the gastrointestinal tract of *Colobus guereza*. In the small intestine, the levels of several metabolites such as 2-aminobutyric acid, 2-aminoethanol, threonine, and tryptophan were higher than those of the other regions.

Discussion

The organic acid concentrations in the forestomach (PS12, TG11, and S10) were higher than those of the Pars Pylorica (PP9), although the profiles of organic acids in PS12, TG11, and S10 were similar to each other (Figure 1B & Table S2). SCFAs, including acetate, propionate, and \( n \)-butyrate were the major organic acids in the forestomach of the *Colobus guereza*, which is consistent with previous observations in the wild colobus [11,12]. In addition, the hindgut digesta contained higher levels of SCFAs, similar to those of the forestomach; however, the ratio of acetate-to-propionate was higher in the hindgut than that in the forestomach (Figure 1B & Table S2). These results indicate that microbial digestion and fermentation of plant materials occurred in both the forestomach and hindgut of the zoo *Colobus guereza* as described previously [13]. Interestingly, high

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**Figure 1A, 1B:** The gastrointestinal tract and organic acid concentrations of the digesta in *Colobus guereza* (A). No.12, presaccus (designated as PS12); No.11, tubus gastricus (TG11); No.10, saccus (S10); No.9, Pars Pylorica (PP9); No.8, duodenum (D8); No.7, proximal jejunum (PJ7); No.6, distal jejunum (DJ6); No.5, ileum (I5); No.4, cecum (C4); No.3, proximal colon (PC3); No.2, distal colon (DC2); No.1, rectum (R1). Orange area, lactate; red area, acetate; yellow area, propionate; purple area, iso-butyrate; light-blue area, \( n \)-butyrate; black area, iso-valerate; and green area, \( n \)-valerate. Succinate and formate were not detected, therefore, we omitted these acids from the graphs.

**Table 1:** Top 10 abundant genus in the stomach of *Colobus guereza*.

| Phylum         | Class            | Order            | Family            | Genus               | PS12 | TG11 | S10 | PP9 | Stomach mean |
|----------------|------------------|------------------|-------------------|---------------------|------|------|-----|-----|--------------|
| Bacteroidetes  | Bacteroidia      | Bacteroidales    | Prevotellaceae    | *Prevotella*        | 43.2 | 38.0 | 28.2 | 12.6 | 30.5         |
| Firmicutes     | Clostridia       | Clostridiales    | Veillonellaceae   | *Selenomonas*       | 24.7 | 26.9 | 22.7 | 2.8  | 19.3         |
| Cyanobacteria  | Chloroplast      | Streptophyta     | unclassified      | *Butyrivibrio*      | 4.5  | 7.5  | 10.1 | 2.0  | 6.0          |
| Firmicutes     | Clostridia       | Clostridiales    | Clostridiaceae    | *Turicibacter*      | 0.0  | 0.0  | 0.0  | 18.3 | 4.6          |
| Firmicutes     | Clostridia       | Clostridiales    | *Lachnospiraceae* | *IV-tegasphaera*    | 3.9  | 3.8  | 4.4  | 2.4  | 3.6          |
| Firmicutes     | Clostridia       | Clostridiales    | Veillonellaceae   | *IV-tegasphaera*    | 5.0  | 4.7  | 2.6  | 0.7  | 3.3          |
| Firmicutes     | Clostridia       | Clostridiales    | Veillonellaceae   | *unclassified*      | 4.4  | 4.8  | 0.6  | 0.1  | 2.4          |
| Firmicutes     | Clostridia       | Clostridiales    | Veillonellaceae   | *Other*             | 3.9  | 3.6  | 1.7  | 0.3  | 2.4          |
| Bacteroidetes  | Bacteroidia      | Bacteroidales    | *p-2534-1B5*     | *unclassified*      | 0.5  | 0.5  | 3.8  | 2.7  | 1.9          |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | *Rhizobiales* | *Rhizobium*         | 0.0  | 0.0  | 0.0  | 7.3  | 1.8          |
concentrations of SCFAs were also detected in the hindgut that may implicate the hindgut in the microbial digestion and fermentation of undigested fibrous feed residues after foregut digestion. Previously, high levels of SCFAs, especially acetate, were detected in in vitro cultures of the feces of zoo Colobus guereza, showing a high acetate-to-propionate ratio [26]. Collectively, Colobus guereza might absorb SCFAs as energy sources in both the forestomach and the hindgut fermentation.

The metagenome analysis revealed that each gastrointestinal digesta had different microbiota. Genera Prevotella and Selenomonas were detected as the dominant genera in the forestomach; Butyrivibrio and Megasphaera were also detected (Table 1). These are popular genera in the rumen microorganisms and play an important role in rumen metabolism [27,28]; therefore, the foregut microbial ecosystem of Colobus guereza may be similar to the rumen ecosystem. Genus Prevotella comprised 42-60 % of the bacterial rRNA gene copies in the bovine rumen [28], which was similar to the abundance of Prevotella in the forestomach of Colobus guereza (Table 1). In the large intestine, the family Ruminococcaceae was the most abundant, although the major genus was not classified (Table 3). Genera Ruminococcus and Oscilllospira were also identified in the rumen [29-31], especially Ruminococcus favefeciens and R. albus are well-characterized cellulolytic rumen bacteria. In a previous metagenome study, Oscillibacter and Faecalibacterium were predominant in the feces of the wild Colobus guereza [15]. These microbiome in wild colobus were different from our metagenome results in the zoo Colobus guereza (Table 3). A previous report also showed that gut microbiota was different between wild and captive monkeys, Rhinopithecus brelichi [32].

In the small intestine and the pars pylorica, lactate was a major product of organic acid (Figure 1B & Table S2); however, genera Lactobacillus and Bifidobacterium were almost undetectable in the small intestine, therefore, other microorganisms might produce lactate in the intestine of Colobus guereza. Obviously, Clostridium was predominant in the small intestine and the pars pylorica (Table 2). Previously, Clostridium thermolacticum was identified as a thermophilic anaerobe producing high amounts of lactate [33]; therefore, Clostridium might be linked to lactate fermentation in Colobus guereza. Furthermore, methane producers, such as Methanosphaera and Methanobrevibacter, were found in the small intestine but not in the foregut. Genera Methanosphaera and Methanobrevibacter were identified in the large intestine of mammals [34-36], and also in the rumen [37]. Methane metabolism in the small intestine of mammals has not been well investigated; therefore, the research about small intestinal methanogens should be focused on to understand the physiology of methane production in the future.

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