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# Analysis of Diversity of Reindeer Rumen Bacteria Involved in the Cellulose Decomposition

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***Abstract*—Bacteria involved in the cellulose decomposition are dominant in the community of microorganisms of the rumen of various ruminants, including reindeer. Reindeer (Rangifer tarandus) is a unique ruminant adapted to live in adverse conditions of meager diet of the Far North. As a result of the study, it was shown that the proportion of bacteria phyla *of Firmicutes* and *Bacteroidetes*, which includes the overwhelming majority of cellulolytic bacteria, comprised from 84.3% to 86.9% of the rumen bacterial community: not more than 4% of *Proteobacteria*; *Cyanobacteria, Spirochaetes, Verrucomicrobia* and *Actinobacteria* – not more than 5.6%, the rest – in a minor amount. Phylum *Bacteroidetes* was dominant among representatives of other phyla; their share ranged from 45.6% to 52.1%. The largest proportion of cellulolytic bacteria was detected in young deers at the age of 0.5 years (52.5%). Animals showed a tendency to decrease in the share of these bacteria with age. The smallest relative abundance of cellulolytic bacteria was detected in animals at the age of 9 years (44.7%). Bacteria of the genus *Prevotella* dominated among cellulolytic rumen bacteria. *Bacteroides sp., Ruminococcus sp., Blautia sp., Clostridium sp., Butyrivibrio sp. and Paraprevotella sp.* ranked next in relative abundance among rumen bacteria. Their share averaged from 3% to 6%. Our analysis showed the presence of two clusters uniting the microbial communities of the reindeer rumen into groups by the age characteristics. The first cluster included individuals under the age of 5 years, and the second group was older than 6 years. This is consistent with the data of biodiversity indices, which showed that bacterial diversity in the reindeer rumen increases with the age.**

***Keywords—cellulolytic bacteria, rumen, biodiversity, Rangifer tarandus, NGS.***

## I. INTRODUCTION

One of the features of ruminants is the presence of microorganisms in the gastrointestinal tract (in particular, in the rumen), which are capable of cellulose decomposing with a large amount plant polymers that are resistant to decomposition (cellulose, hemicellulose, xylans, starch, and others). The symbiosis between the bacterial community and ruminants arose as a result of evolution, allowing ruminants to efficiently use the energy of plant feed.

In the plant cell walls, cellulose fibers are embedded in a matrix of hemicellulose (mainly xylan, but also mannan, xyloglucan and β-glucan), lignin and pectin. The cellulosic component is crystalline and insoluble. Cellulose becomes even more resistant to degradation in its natural state when cellulose fibers are physically embedded in a matrix of hemicellulose and lignin [1]. Many enzymes (for example, cellulases and hemicellulases) are responsible for the cellulose decomposition, which are grouped into 150 families of different hydrolases, classified according to their sequence, function, and structural properties [2]. For the effective degradation of plant fiber, the coordinated action of several hydrolases acting on different parts of a complex biopolymer is required.

Microorganisms form a rich and diverse symbiotic community in the rumen, which contains up to $10^{11}$ bacteria/ml, $10^3$–$10^7$ fungi/ml, and $10^9$ archaea/ml, $10^6$ protozoa/ml, which are in constant interaction. A key role in the community is played by microorganisms capable of degrading cellulose and hemicellulose. They were the first to colonize plant residues, breaking down bonds inside cellulose and hemicellulose and releasing substances more accessible for assimilation by heterotrophic bacteria (glucose, cellobiose, etc.).

The composition of the microbial community is influenced by a large number of factors. The studies revealed a number of characteristic patterns of the species composition of the rumen microbiota depending on the genotype of animals [3], their age [4], habitat [5], season [6-7], diet and feeding regimen [8], health [9-10], antibiotics use [9], daylight hours [11], stress [12] and environment [13].

Rumen cellulosolytic bacteria are mainly related to the phyla of *Firmicutes* and *Bacteroides* [3]. Representatives of these bacterial phyla dominate the rumen microbial, including in reindeer. Modern methods of analysis have made it possible to establish not only the species composition of the rumen bacteria consortium at a deep level, but also to understand their enzymatic potential. The circle of bacteria capable of participating in the cellulose decomposition in the rumen has expanded. To date, the bacteria allegedly involved in the primary cellulose decomposition in the rumen include such genera as *Prevotella, Fibrobacter, Ruminococcus, Butyrivibrio, Bacteroides, Ruminobacter, Treponema, Selenomonas and Clostridium*. According to the studies, regardless of the type of fiber, *Prevotella sp., Butyrivibrio sp. and Ruminococcus sp*. dominated the majority of the community, which indicates that these taxa have extensive potential for the synthesis of a wide range of hydrolases [14].

Reindeer (Rangifer tarandus) is a unique ruminant adapted to live in adverse conditions of the Far North. A poor diet, especially in the winter-spring period, and adverse living

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conditions, undoubtedly exert significant breeding pressure on the structural and functional organization of rumen microbiome and the community of cellulose decomposing bacteria inside it. Reindeer wintertime diet consists of 70% of lichens, which are very toxic for many animals, for example, sheep and cows due to the content of usnic acid, a toxic metabolite of lichens.

The study of the compositional characteristics of such a physiological group of bacteria as cellulolytic in the rumen of reindeer of various ages will contribute to a deeper understanding of the processes of cellulose decomposition that occur in the rumen. This article will be the first to analyze the cellulolytic bacteria community in the reindeer rumen of the Nenets Autonomous Okrug and its dynamics during ontogenesis. The aim of this study was to analyze the rumen community of cellulolytic bacteria of reindeer (Rangifer tarandus) and to identify patterns of change in its composition during animal ontogenesis.

## II. Materials and methods

During the winter of 2017, an expedition was organized to the territory of the Nenets Autonomous Okrug in the area of the Nelmin-Nos village; rumen content samples from reindeers of various ages were selected (from six-month-old young animals to 3, 5, 6 and 9 year old). Samples of rumen fluid were taken from three animals of each age. A total of 15 samples of rumen content were selected.

Immediately after selection, samples were frozen at a temperature of -20°C, and then placed for long-term storage in a freezer at a temperature of -70°C.

Isolation of total DNA for molecular biological analyzes was carried out according to the procedure described by Maniatis et al. in its own modification [15].

The bacterial rumen community was evaluated by NGS sequencing on the next generation sequencing platform MiSeq (Illiumina, USA) using reagents for preparing libraries of Nextera® XT IndexKit, for refining of PCR products – Agencourt AMPure XP, for sequencing – MiSeq® ReagentKit v2 (500 cycle). Processing of the resulting reads, including overlapping, quality filtration (Q30), and primer trimming, was performed using the Illumina bioinformatics platform. Determination of the taxonomic affiliation of microorganisms was performed in the 16S Metagenomics application of the MiSeq Reporter software using the GreenGenes taxonomic database using the Ribosomal Database Project (RDP) classifier algorithm.

Statistical processing, calculation of Shannon's and Simpson's biodiversity indices, was carried out using Microsoft Excel 2010 and Past software. Cluster analysis was performed according to the Bray-Curtis dissimilarity in the Past program.

## III. Results and discussion

The morphological features of the digestive system in various ruminants [16-17] suggest the formation of unique microbial rumen communities with a wide spectrum of enzymatic activity. Cellulose decomposition is the main function of the microbial community in the rumen, which allowed ruminants to cover many habitats with a wide range of climatic conditions [18] over the course of evolution, lasting millions of years. In turn, the host organism creates optimal conditions for the life of microorganisms, i.e. for their growth and reproduction (by nutrients, ambient temperature, and buffer composition of the environment [19].

Wu et al. [20] described a crustal rumen microbiome that included 8 phyla (*Bacteroidetes, Firmicutes, Proteobacteria, Spirochaetes, Fibrobacteres, Verrucomicrobia, Synergistetes, and Actinobacteria*). However, the vast majority of the microbiome (up to 90%) is occupied by representatives of phyla of *Bacteroidetes and Firmicutes* [21].

As a result of the analysis of NGS sequencing, it was shown that 27 phyla were identified in the bacterial community (Fig. 1), including 25 bacterial and 2 archeological ones. The total percentage of bacteria phyla of *Firmicutes and Bacteroidetes* in the rumen bacterial community comprised 84.3% to 86.9%; *Proteobacteria* not more than 4%; *Cyanobacteria, Spirochaetes, Verrucomicrobia and Actinobacteria* not more than 5.6%, the rest – in minor quantities. Phylum *Bacteroidetes* dominated among representatives of other phyla; their share ranged from 45.6% to 52.1%. Furthermore, the largest share of this phylum was detected in animals of 3 to 5 years of age. The relative number of representatives of the *Firmicutes* phylum, on the contrary, reached a maximum in young six-month-old animals and adults at the age of 9 years.

Community ecological biodiversity indices had some differences among reindeer of different ages. The intrinsic diversity of the communities was characterized using the Shannon's and Simpson's alpha diversity indices.

The Shannon Index, which characterizes biodiversity and alignment of communities, ranged from 3.3 to 3.5. The Shannon Index was higher in rumen communities of reindeers between 5 and 9 years old. Thus, with age, the community becomes more diverse. The Simpson's dominance index ranged from 0.902 to 0.937. At the age of 6-9 years, the indices reach their maximum values, indicating that in these bacterial communities of the rumen, dominance was more pronounced.

TABLE I. Ecological biodiversity indices of bacterial communities in reindeer rumen in the Nenets Autonomous Okrug

| **Indicies** | **Age of animals** | | | | |
|---|---|---|---|---|---|
| | ***0,5 year*** | ***3 years*** | ***5 years*** | ***6 years*** | ***9 years*** |
| Genera quantity | 224±15 | 228±4 | 239±15 | 235±8 | 225±11 |
| Simpson (D) | 0.923 ± 0.031 | 0.904 ± 0.026 | 0.922 ± 0.035 | 0.937 ± 0.040 | 0.935 ± 0.041 |
| Shannon (H) | 3.360 ± 0.151 | 3.301 ± 0.112 | 3.434 ± 0.145 | 3.479 ± 0.158 | 3.464 ± 0.147 |

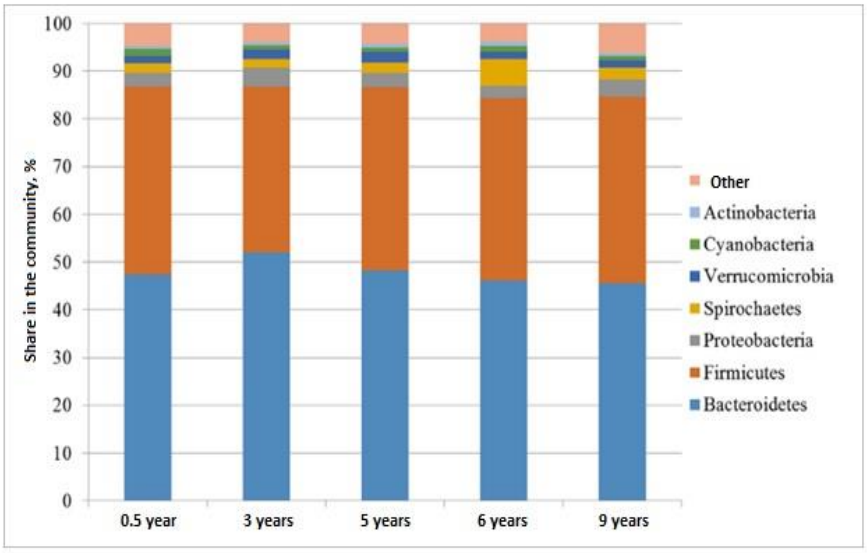


Fig. 1. Taxonomic diversity of bacteria community in reindeer rumen in the Nenets Autonomous Okrug by phyla

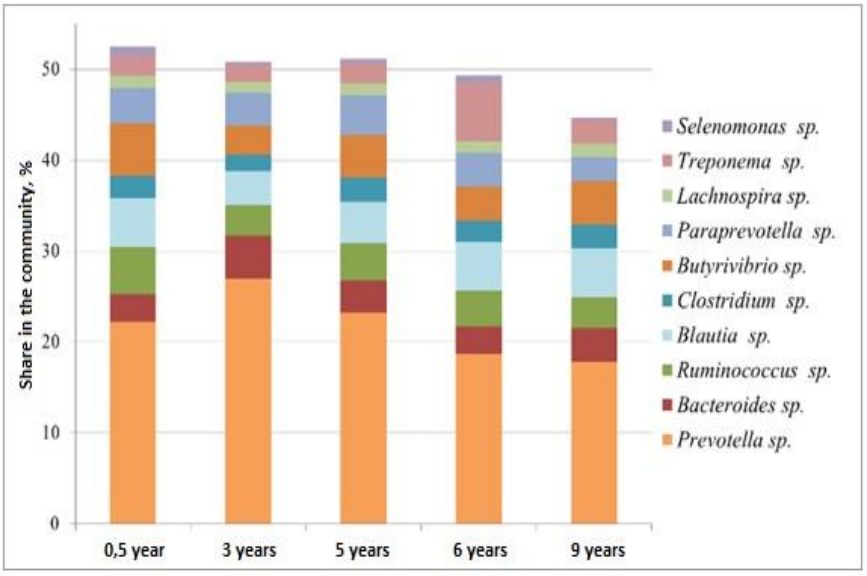


Fig. 2. Relative number of bacteria involved in the cellulose decomposition in reindeer rumen in the Nenets Autonomous Okrug

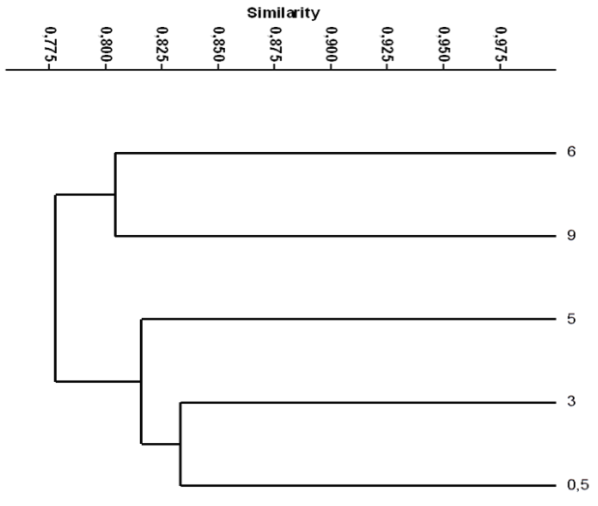


Fig. 3. Cluster analysis of bacterial communities of reindeer rumen in the Nenets Autonomous Okrug by different ages

Rumen cellulolytic bacteria are mainly related to the phyla of *Firmicutes and Bacteroides* [3]. Representatives of these bacterial phyla dominate the rumen microbial community, including in reindeer. To date, the bacteria presumably involved in the primary cellulose decomposition in the rumen include such genera as *Prevotella, Fibrobacter, Ruminococcus, Butyrivibrio, Bacteroides, Ruminobacter, Treponema, Selenomonas, and Clostridium* [14].

The largest proportion of cellulolytic bacteria of the community (Fig. 2) was detected in young deer at the age of 0.5 years (52.5%). Animals showed a tendency to decrease in the share of these bacteria with age. The smallest relative abundance of cellulolytic bacteria was detected in animals at the age of 9 years (44.7%). Bacteria of the genus *Prevotella* dominated among representatives of this physiological group and the rumen bacteria community. This is consistent with the data of a number of authors, who showed that, regardless of the type of fiber, bacteria of the genera *Prevotella* and *Butyrivibrio* dominated the community in majority of the studies, which indicates that these taxa have great potential for

the synthesis of a wide range of hydrolases [22-23]. On average, *Preotellians* occupied about 20% of the total community, reaching the greatest abundance by age 3 (27%) to 5 years (23%). Subsequently, by the age of 6-9 years, the number of *Prevotella sp*. decreased to 18%.

The role of *Prevotella* in the processes of fiber decomposition was revealed not so long ago. In the phylogenetic analysis, the fiber-related bacterial rumen community, large clusters associated with *Prevotella sp.* sequences were extracted from in situ incubated fiber in the sheep rumen, which implies the possible involvement of *Prevotella sp.* in fiber decomposition [24].

There are studies showing that a number of bacteria, including *Prevotella*, can take part in the decomposition of not only cellulose and hemicellulose, but also lignin [25].

*Bacteroides sp., Ruminococcus sp., Blautia sp., Clostridium sp., Butyrivibrio sp. and Paraprevotella sp*. ranked next in relative abundance among rumen bacteria. Their share averaged from 3% to 6%. For the genus *Bacteroides* bacteria, the same pattern was characteristic as for *Prevotellas*. They reached the highest relative abundance (3.5 -5%) in middle age. While in young animals and older individuals, the share of these bacteria decreased to 3%. In bacteria *Ruminococcus sp., Blautia sp. and Butyrivibrio sp.* the reverse pattern is noted. Their number reached minimal values in animals of 3-5 years of age.

The relative number of bacteria of the genus *Clostridium* changed insignificantly in the community of reindeer rumen microorganisms, occupying on average 2.0-2.5% of the total community. Share of *Butyrivibrio sp.*is independent of the reindeer age. The relative abundance of these bacteria underwent fluctuation changes throughout animals' life.

Bacteria belonging to the genus *Selenomonas* did not exceed 1% in the reindeer rumen bacteria community. They have some hydrolytic activity, and, according to recent studies, they are ones of the first microorganisms to colonize cellulose fibers in the rumen [14]. The relative abundance of these bacteria in the reindeer rumen reached maximum in young six-month-old individuals.

*Fibrobacter* bacteria (*Fibrobacteres* phylum) are widely known as rumen cellulose destructors [26]. There are plenty of these bacteria found in cattle. Our analysis of the microbial community of the reindeer rumen of the Nenets Autonomous Okrug practically did not reveal any bacteria of the *Fibrobacter* genus. In general, this is consistent with the data obtained as a result of the analysis of rumen microbiomes of animals belonging to *Cervidae* family (reindeer and elk), in which the *Fibrobacteres* phylum bacteria did not occupy a dominant position compared to cattle, in which this phylum often became dominant [21].

The cluster analysis (Fig. 3) showed the presence of two clusters uniting the microbial communities of the reindeer rumen into groups by the age characteristics. The first cluster included individuals under the age of 5 years, and the second – over 6 years old. This is consistent with the data of biodiversity indices, which showed that bacterial diversity in the deer rumen increases with age.

It was previously shown that the key bacteria that decompose fibers appear in the rumen just a few days after birth, and the rumen's ecosystem actually functions in the way of cellulose decomposition at such an early age [27]. It was also shown that the structure of the rumen microbiome (composition and abundance) stabilizes at the age of 2 to 6 months, regardless of the diet of animals [28]. Apparently, our study has shown that with age, further development of the rumen community occurs, a greater variety of bacteria accumulates, and rearrangements in the relative number of dominant species in the community occur.

## IV. Conclusions

Our results led to conclusions about the composition of cellulolytic bacteria community in reindeer rumen of the Nenets Autonomous Okrug during ontogenesis. The proportion of *Firmicutes and Bacteroidetes* phyla bacteria, which includes the overwhelming number of bacteria that decompose cellulose in the rumen, amounted from 84.3% to 86.9% in the rumen bacterial community. Phylum *Bacteroidetes* dominated among representatives of other phyla; their share ranged from 45.6% to 52.1%.

The largest proportion of cellulolytic bacteria was detected in young deer at the age of 0.5 years (52.5%). Animals showed a tendency to decrease in the share of these bacteria with age. The smallest relative abundance of cellulolytic bacteria was detected in animals at the age of 9 years (44.7%). Bacteria of the *Prevotella* genus dominated among representatives of this physiological group and the rumen bacteria community. On average, *Prevotella sp*. occupied about 20% of the total community, reaching the largest numbers by the age of 3 (27%) and 5 years (23%). Subsequently, the abundance of *Prevotella sp*. decreased to 18%. In addition to *prevotellas*, other cellulolytic bacteria were identified in the rumen – their share was from 3% to 6%: *Bacteroides sp., Ruminococcus sp., Blautia sp., Clostridium sp., Butyrivibrio sp. and Paraprevotella sp.*

When analyzing the reindeer rumen microbial community in the Nenets Autonomous Okrug, bacteria of the *Fibrobacte*r genus (one of the most common cellulose-decomposing rumen bacteria) were practically not detected.

The analysis showed the presence of two clusters uniting the microbial communities of the reindeer rumen into groups by the age characteristics. The first cluster included individuals under the age of 5 years, and the second – over 6 years old. This is consistent with the data of biodiversity indices, which showed that bacterial diversity in the deer rumen increases with age.

Hence, there are a number of changes in the bacterial community of the reindeer rumen, associated with the accumulation of greater species diversity during ontogenesis, a decrease in the relative number of cellulolytic bacteria and the preservation of the core microbiome of cellulolytic bacteria during the entire ontogenesis, which undergoes some fluctuations.

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