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Search and Isolation of Microorganisms from the *Rangifer Tarandus* Rumen Possessing Mycotoxin Biodegradation Potential and Cellulolytic Activity

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***Abstract*—Rumen content of the *Rangifer tarandus* is a promising source for the search for bacteria with high biological activity. Our objective was to screen isolates with high biological activity from the rumen content of reindeer in the Nenets and Yamalo-Nenets Autonomous Okrug of Russia. A total of 63 associations of microorganisms were investigated. Most of the studied associations showed the ability to decompose cellulose in the form of filter paper on the 3rd-6th day of incubation. 52 isolates demonstrated decomposition properties of carboxymethyl cellulose (according to data on the 3rd day of incubation). The results of the analysis of the cellulolytic activity of ten isolates that were selected as a result of screening according to the method of Henderson, Horvath and Block in the Churlis modification showed the level of cellulose decomposition from 4.0±0.34 to 62.0±4.1%. Four promising isolates selected at the next stage had the ability to biodegrade at least three mycotoxins. The highest level of biodegradation was observed for aflatoxins and reached 75.1±3.9%. The obtained experiments led to the conclusion that the isolates of microorganisms isolated from the reindeer rumen are capable of synthesizing cellulases and enzymes that biodegrade mycotoxins, which probably allows them to gain a competitive advantage in the rumen of a deer that consumes diets with an abundance of indigestible fiber and secondary metabolites with toxic properties. These strains are of undoubted interest for livestock (including dairy cattle and reindeer husbandry) with the aim of using them as environmentally friendly and safe probiotic feed additives.**

Keywords—rangifer tarandus, rumen, mycotoxin biodegradation, cellulolytic microorganisms.

I. INTRODUCTION

When developing preventive and therapeutic veterinary medication, it should be borne in mind that the basis of the modern concept of animal husbandry is environmental safety, including the search for natural biological agents that are alternative to antibiotics and drugs.

A promising source for the search for bacteria with high biological activity is the rumen content of ruminant animals. A feature of representatives of cicatricial ruminant microbiocenosis, which evolves together with a macroorganism, is the ability to form a number of digestive enzymes, including cellulases, which allows ruminants to effectively use the energy of feeds rich in fiber. Reindeer (Rangifer tarandus) is a unique representative of ruminants, which has acquired specific adaptations for life in the North. Thus, in the diet of reindeer, the proportion of lichens containing significant amounts of indigestible polysaccharides: chitin, hemicellulose, etc., reaches up to 70%. In this regard, unlike cattle bred in conditions of intensive animal husbandry, deer cellulolytic rumen microorganisms play a decisive role in digestion.

In addition, lichens are active producers of more than 250 secondary metabolites. First of all, these are lichen acids (salicylic, usnic, lecanoric, etc.). Usnic acid [1], which is produced by lichens of the genera *Cladonia, Usnea, Lecanora, Ramalina, Evernia, Parmelia, Alectoria* and others, is the most famous secondary metabolite with toxic properties.

There are a number of studies [2-4], where the researchers proved the presence of mycotoxins in combination in various species and genera of lichens. For example, Burkin and Kononenko [4] revealed the presence of zearalenone in the lichens of *Cladonia and Nephroma* in amounts of up to 90 and 150 ng/g, respectively, as well as a number of other mycotoxins. Later, Burkin and Kononenko [5] using the enzyme-linked immunosorbent assay described the occurrence and frequency of mycotoxin accumulation in lichens belonging to 20 genera of the families *Cladoniaceae, Nephromataceae, Parmeliaceae, Peltigeraceae, Teloshisthistaeae and Umbilicariacece*. Alternariol, sterigmatocystin, mycophenolic acid, citrinin, cyclopiazonic acid, and emodin with a content of more than 1000 ng/g, i.e. 0.0001%, were found in all genera except *Peltigera*. As a matter of interest, land plants in the composition of reindeer diets are also affected by combinations of mycotoxins to a large extent, as was shown by us previously [6]. In a significant number of samples of higher plants, *Salix borealis, Vaccinium uliginosum, Betula nana, B. pendula*, as well as a mixture of various perennial grasses growing in the territories of tundra and forest-tundra pastures located in the Arctic regions of Russia, T-2 mycotoxin, ZEN (zearalenone) and DON (deoxynivalenol) were present produced by pathogens of fusarium, which affects vegetative plants, as well as aflatoxins and ochratoxin A, metabolites of *Aspergillus sp., Penicillium sp*. micromycetes, previously considered not adapted for growth and reproduction on plant tissues during the growing season.

Close attention of researchers in recent years has been attracted by the ability of microorganisms to carry out

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biodegradation of bacterial and fungal toxins to non-toxic compounds [7-9] with the aim of developing highly effective biological products. It is assumed [10-11] that the efficiency of the biotransformation of toxins is ensured by the large lability of microorganisms' metabolism: a huge variety of enzyme systems, synthesis of organic acids and other various compounds. According to some researchers, bacteria associated with deer rumen can actively detoxify usnic acid [12-14] and mycotoxins [2, 4-5] contained in components and diets.

In this regard, our objective was to screen highly effective isolates from the reindeer rumen content in order to create a collection of bacteria with cellulolytic properties and the ability to biodegrade mycotoxins.

II. MATERIALS AND METHODS

In order to isolate microorganisms' isolates from the contents of reindeer rumen, we organized expeditions in 2017 to the regions of the Russian Arctic that are part of various climatic zones: the Nelmin-Nos village of the Nenets Autonomous Okrug (tundra natural-climatic zone), and the Harp village of the Yamal-Nenets Autonomous Okrug (forest-tundra natural-climatic zone) (Fig. 1).

The contents of the reindeer rumen were selected from a clinically healthy male of 6 years old and a calf of 5 months (living in the territory of the Kharp village), as well as a male of 4 years old and a female of 7 years old (living in the Nelmin-Nos village of the Nenets Autonomous Okrug).

Fig. 1. Expedition to the Nelmin-Nos village of the Nenets Autonomous Okrug (2017)

The taxonomic composition of the autumn-summer diets of reindeer [15] of the Nenets and Yamalo-Nenets Autonomous Okrug is presented in Table I. The nutritional value of multicomponent samples repeating the composition of the average summer pasture diet of reindeers is presented in Table II.

TABLE I. TAXONOMIC (BOTANICAL) COMPOSITION OF AUTUMN-SUMMER DIETS OF REINDEER, % [15].

Dietary component	**Component share in total diet, %**	
	Kharp village	***Nelmin-Nos (tundra)***
Reindeer lichen brushwood		
Cladonia Lichens	5	10
Nephroma Lichens	5	-
Trees		
Silver birch (Betula pendula)	5	20
Shrubs		
Pollar willow (Salix polaris)	15	-
Northern willow (Salix borealis)	5	20
Smaller shrubs		
Bog blueberry (Vaccinium uliginosum)	10	-
Dwarf birth (Betula nana)	25	20
Perennial herbs		
Perennial herbs blend	30 *Poa arctica, Calamagrostis epigeios, Tanacetum vulgare, Galium boreale*	30 *Poa arctica, Eriophorum vaginatum, Alopecurus pratensis*

TABLE II. NUTRITION OF MULTICOMPONENT SAMPLES THAT REPEAT THE COMPOSITION OF THE AVERAGE SUMMER PASTURE DIET OF REINDEERS (M±M, N=3)

№	**Indicator, units of measurement**	**Content**	
		Harp village	***Nelmin-Nos village***
1	Mass fraction of easily hydrolyzable carbohydrates, %	0.54±0.47	1.83±0.6
2	Mass fraction of soluble carbohydrates, %	2.72±0.57	8.15±1.03
3	Feed units in natural matter, F. units/kg	0.59±0.032	0.64±0.045
4	Feed units in dry matter, F. units/kg	0.74±0.042	0.76±0.039
5	Mass fraction of dry matter, %	76.74±1.6	82.04±1.46
6	Mass fraction of crude fat in natural substance, %	2.2±0.25	1.88±0.77
7	Mass fraction of crude protein in natural substance, %	12.5±0.4	7.81±0.16
8	Mass fraction of crude ash in natural substance, %	4.75±0.23	2.28±0.19
9	Mass fraction of crude fiber in natural substance, %	18.61±1.9	19.51±1.9
10	Metabolic energy in a natural substance, MJ/kg	7.49±0.49	8.06±0.5
11	Exchange energy in dry matter, MJ/kg	9.44±0.63	9.51±0.94

Sampling of rumen content was carried out with a sterile probe in compliance with aseptic conditions in the Yamal-Nenets Autonomous Okrug – in the reindeer herding team of the Yamal Department of the All-Russian Research Institute of Veterinary Entomology and Arachnology; the Nenets Autonomous Okrug – in the reindeer herding team of agricultural production cooperative "ERV".

Transportation of samples was carried out in thermal containers at +6°C in order to maintain the viability of microorganisms (no more than 1-2 days).

To identify associations (isolates) of bacteria with high cellulase activity, 0.5 ml deer rumen fluid samples were cultured on the following medium: GRM nutrient broth (State Scientific Center for Applied Microbiology and Biotechnology, Russia) – 0.5 l, distilled water – 0.5 l, CaCO3 – 0.5%, filter paper (2-3 strips, 0.5x3 cm) in 10 ml tubes at +37 °C. The degree of decomposition (integrity) of filter paper was evaluated in dynamics on the 3rd, 4th, 5th, 6th day [16].

To analyze the level of decomposition of carboxymethyl cellulose, weighed portions of rumen fluid (0.5 g each) were suspended with 50 ml of a 0.85% (weight/volume) sterile NaCl solution in a conical flask, which was rotated at 180 rpm for 1 hour at 37 °C. Serial dilutions were prepared up to 10-7 using sterilized saline. An aliquot of 100 µl of each of the solutions was added to Bushnell Haas medium with the addition of carboxymethyl cellulose (pH = 7.0) containing (g/l): carboxymethyl cellulose (10.0), K2HPO4 (1.0), KH2PO4 (1.0) MgSO4·7H2O (0.2), NH4NO3 (1.0), FeCl3·6H2O (0.05), CaCl2 (0.02) and agar (20.0). Petri dishes were incubated at +37 °C. The decomposition level of carboxymethyl cellulose was evaluated on a solid nutrient medium on the 3rd day.

Associations of microorganisms were stored in oil under anaerobic conditions at t = +4°C.

The exact level of cellulolytic activity of the most active associations was analyzed by the Henderson, Horvat, and Block method in the Churlis modification, which is based on determining the difference in the weight of cellulose (filter paper) before and after its incubation with the culture of microorganisms in vitro [17-18]. The culture of the isolated associations was introduced in triplicate of 10 ml into tubes with filter paper, dried to constant weight at a temperature of +104-106 °C and in medium of the following composition: NaH2PO4 – 2041.6 mg/l, NaHCO3 – 2041.6 mg/l, KCl – 262.4 mg/l, KJ – 28.03 mg/l, NaCl – 262.4 mg/l, MgSO4 – 8.5 mg/l, FeSO4·7H2O – 31.2 mg/l, MnSO4 – 15.6 mg/l, CuSO4·5H2O – 1.6 mg/l, ZnSO4·7H2O – 0.47 mg/l, CoSO4 – 0.7 mg/l, Na2P4O7·10H2O – 0.07 mg/l, CrCl2·5H2O – 0.16 mg/l, K2CrO4 – 0.08 mg/l, NaAsO3 – 0.13 mg/l, glucose 500 mg/l, urea 840 mg/l, distilled water – 1 l. Cultivation was carried out in a thermostat for up to 2 weeks at a temperature of +37 °C. Then the filter was removed, washed and dried to constant weight at a temperature of +104-106 °C. Afterwards we weighed and calculated the amount of decomposed cellulose in the sample.

The ability of the most active cellulose-degrading isolates to transform mycotoxins was analyzed. At the first stage of the study, we studied the resistance (viability) of the isolated isolates to the action of mycotoxins in in vitro experiments with the cultivation of microorganisms with different concentrations of mycotoxins introduced into the nutrient medium of the following composition: nutrient broth (Russian State Scientific Center for Applied Microbiology and Biotechnology, Russia) - 0.5 l, distilled water - 0.5 l, CaCO3 - 0.5%, filter paper (2-3 strips, 0.5 x 3 cm), AFL mycotoxins (aflatoxins) – 12 µg/l, OTA (ochratoxin A) – 15 µg/l, T-2 (T-2 mycotoxin) – 60 µg/l, ZEN (zearalenone) – 100 µg/l or DON (deoxynivalenol) – 1000 µg/l, depending on the type of experiment. The incubation time was 3 days at a temperature of +38 °C in a thermostat with regular stirring.

Using the ELISA method (test systems AgraQuant, Austria, Romer Labs, Inc.), the decrease in the amount of mycotoxin in the solution was determined by the formula:

$$\text{mycotoxin biodegradation}(\%)\frac{(C_{\text{initial}} - C_{\text{final}})}{C_{\text{initial}}} \cdot 100\% \quad (1)$$

Where $C_{initial}$ – the initial concentration of mycotoxin in solution before incubation, µg/l, C_{final} – the residual concentration of mycotoxin in the solution after joint incubation with bacteria, µg/l.

In order to perform ELISA, the extract of the sample (or standard) and conjugated with the enzyme mycotoxins were mixed, then introduced into the microwells containing antibodies, washed and the enzyme substrate was added. The intensity of the staining of the substrate was inversely proportional to the concentration of mycotoxin in the sample or standard. Next, stopping solutions were added: 10% hydrochloric acid in the case of ZEN and T-2 mycotoxin and 10% phosphoric acid in the case of the rest of the analyzed mycotoxins.

The optical density was measured at λ = 450 nm using a Stat Fax 303+ microstrip photometer (Awareness Technology, Inc., USA), comparing the values for the sample and for standards.

Mathematical and statistical processing of the results was carried out by standard methods of analysis of variance [19] using EXCEL XP/2010 software.

III. RESULTS AND DISCUSSION

As noted by several researchers [20-21], in the complex process of splitting polymer feed substrates into monomers and the subsequent synthesis of VFA, vitamins and bacteriocins, the rumen microorganisms do not participate separately, but together, in the form of a microbial consortium, where cross-transfer of nutrients takes place. In this regard, we have identified multicomponent associations of microorganisms from the reindeer rumen content. A total of 63 associations of deer rumen microorganisms were investigated.

The results showed that most of the studied associations showed the ability to decompose cellulose on the 3rd-6th day of incubation (Table III, Fig. 2). However, the level of cellulose decomposition was expressed differently for each isolate. Only 6 studied associations demonstrated the possibility of cellulose decomposition already on the 3rd day of incubation. Most isolates (35 of the all studied ones) showed the ability to decompose cellulose on the 4th day of incubation.

52 isolates (including the most active in the decomposition of filter paper) demonstrated the decomposition properties of carboxymethyl cellulose (according to the data on the 3rd day of incubation) (Table III).

TABLE III. ANALYSIS RESULTS OF THE SELECTED RUMEN ISOLATES' ABILITY TO DECOMPOSE CELLULOSE (IN THE FORM OF FILTER PAPER)

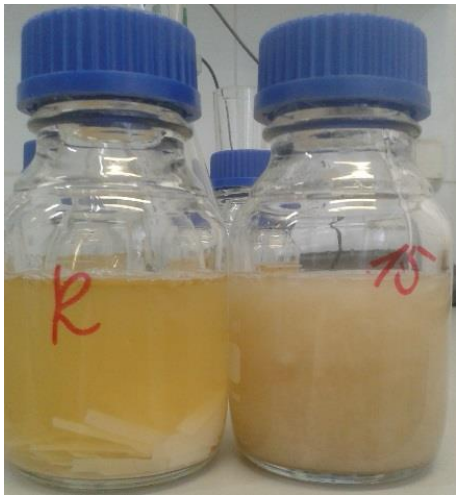
Isolate collection number	Cellulose decomposition level on 3rd-6th day of incubation				Ability to decompose carboxymethyl cellulose on 3rd day of incubation
	Day 3	*Day 4*	*Day 5*	*Day 6*	
Yamal-Nenets Autonomous Okrug (stag, 6 years of age)					
1	-	-	+*	++**	yes
2	-	+	++	++	yes
3	-	-	-	-	no
4	-	+	++	+++***	yes
5	-	+	++	+++	no
6	-	+	++	+++	yes
7	+	++	+++	+++	yes
8	-	+	++	+++	yes
9	-	+	++	+++	yes
10	-	+	++	+++	yes
11	-	-	-	-	no
12	-	-	+	++	yes
13	-	-	-	-	no
14	-	+	++	+++	yes
15	-	+	++	+++	yes
16	-	+	++	+++	yes
Yamal-Nenets Autonomous Okrug (fawn, 5 months)					
17	-	+	++	+++	yes
18	-	+	++	+++	yes
19	-	+	++	+++	yes
20	-	+	++	+++	yes
21	+	+	++	+++	yes
22	-	-	-	+	yes
23	-	-	+	++	yes
24	-	+	++	+++	yes
25	-	-	-	-	no
26	+	++	+++	+++	yes
27	-	-	+	++	yes
28	-	+	++	+++	yes
29	-	+	++	+++	yes
30	-	-	-	-	no
31	-	-	+	+	yes
Nenets Autonomous Okrug (stag, 4 years)					
32	-	-	+	++	yes
33	-	-	+	++	yes
34	-	-	-	+	yes
35	-	-	+	++	yes
36	-	-	+	++	yes
37	-	+	+	++	yes
38	-	-	-	+	no
39	-	+	++	+++	yes
40	-	+	++	+++	yes
41	-	+	++	+++	no
42	-	+	++	+++	yes
43	-	+	++	+++	yes
44	-	+	++	+++	yes
45	-	-	-	-	no
46	+	++	+++	+++	yes
47	-	-	-	+	yes
48	-	-	+	++	yes
Nenets Autonomous Okrug (doe, 7 years)					
49	-	-	-	+	yes
50	-	-	-	+	yes
51	+	++	+++	+++	yes
52	-	-	-	-	no
53	-	-	+	++	yes
54	-	+	++	+++	yes
55	-	+	++	+++	yes
56	-	+	++	+++	yes
57	-	+	++	+++	yes
58	+	++	+++	+++	yes
59	-	-	-	+	yes
60	-	-	-	+	yes
61	-	-	-	-	no
62	-	-	+	++	yes
63	-	+	++	+++	yes

* + is the low level of cellulose decomposition (less than 30%)

** ++ is the medium-pronounced cellulose decomposition (30-70%)

*** +++ is the complete or almost complete cellulose decomposition (more than 70%)

A



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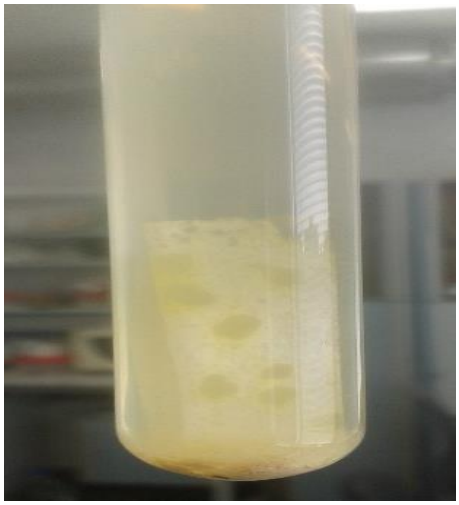

Fig. 2. A, B. In vitro cellulose digestion experiment with isolates of microorganisms

The obtained data seem quite logical, since cellulose is a significant component of the diet of ruminants, especially reindeer; a wide range of bacteria, micromycetes and protozoa possess cellulolytic enzymes in the rumen. Among bacteria, the typical inhabitants of the rumen with cellulases are *Fibrobacter succinogenes*, which transforms cellulose to succinate, acetate, formate [22], *Ruminococcus albus* [23], *Butyrivibrio fibrisolvens* [24], etc.

In our experiment, the isolated associations were visually white and creamy, small (0.5-1 mm), lenticular colonies on a dense medium with carboxymethyl cellulose. Presence of several types of cells was observed during isolates microscopy: small cocci (as well as diplococci and streptococci), small and large bacilli.

The most promising 10 isolates were selected out of the 63 (No. 7, 14, 15, 21, 24, 26, 37, 46, 51, 58). The results of the analysis of the cellulolytic activity of the selected ten isolates by the method of Henderson, Horvath, and Block in the

Churlis modification showed the level of cellulose decomposition from 4.0±0.34 to 62.0±4.1% (Table IV).

TABLE IV. ANALYSIS RESULTS OF ISOLATES' CELLULOLYTIC ACTIVITY BY THE METHOD OF HENDERSON, HORVATH AND BLOCK IN THE MODIFICATION OF CHURLIS (M±M, N=3)

Isolate collection number	Amount of decomposed cellulose, %
7	4.0 ± 0.34
14	44.0 ± 3.52
15	47.8 ±3.31
21	56.8 ±4.7
24	17.8 ±1.53
26	62.0 ±4.1
37	9.5 ±0.62
46	6.0 ±0.32
51	10.0 ±0.83
58	25.5 ±1.9
Bacteria-free culture medium (negative control)	0

According to the results of the screening, isolates numbered 14, 15, 21, 26 showed the highest level of decomposition of original cellulose – from 44.0±3.52 to 62.0±4.1%. In this regard, we evaluated the ability of these isolates to carry out biodegradation of mycotoxins.

The results of incubation of isolates No. 14, 15, 21, 26 isolated from deer rumen fluid in a medium containing mycotoxins are presented in Table 5. The cultivation of bacterial isolates in a liquid nutrient medium containing mycotoxins did not lead to significant growth inhibition in almost all cases microorganisms (Table V).

TABLE V. CHANGE IN THE ISOLATES' TITER DURING CULTIVATION ON A MEDIUM SUPPLEMENTED WITH MYCOTOXINS (CMC AGAR NUTRIENT MEDIUM) (M±M, N=3)

Isolate collection number	Option	AFL	OTA	T-2	ZEN	DON	Negative control (culture medium without mycotoxins)
14	Before incubation	$8.9\times10^8\pm4.2\times10^7$	$8.9\times10^8\pm4.2\times10^7$	$8.9\times10^8\pm4.2\times10^7$	$8.9\times10^8\pm4.2\times10^7$	$8.9\times10^8\pm4.2\times10^7$	$8.9\times10^8\pm4.2\times10^7$
	3rd day of incubation	$5.0\times10^7\pm3.4\times10^6$	$8.7\times10^8\pm5.3\times10^7$	$3.1\times10^8\pm4.2\times10^7$	$9.1\times10^8\pm8.8\times10^7$	$2.7\times10^8\pm1.1\times10^7$	$1.1\times10^9\pm1.2\times10^8$
15	Before incubation	$3\times10^8\pm1.6\times10^7$	$3\times10^8\pm1.6\times10^7$	$3\times108\pm1.6\times10^7$	$3\times108\pm1.6\times10^7$	$3\times108\pm1.6\times10^7$	$3\times108\pm1.6\times10^7$
	3rd day of incubation	$3.3\times10^8\pm1.1\times10^7$	$2.1\times10^8\pm1.4\times10^7$	$2.0\times10^8\pm1.2\times10^7$	$4.3\times10^8\pm3.8\times10^7$	$3.6\times10^8\pm3.3\times10^7$	$4.7\times10^8\pm5\times10^7$
21	Before incubation	$2.7\times10^9\pm1.2\times10^8$	$2.7\times10^9\pm1.2\times10^8$	$2.7\times10^9\pm1.2\times10^8$	$2.7\times10^9\pm1.2\times10^8$	$2.7\times10^9\pm1.2\times10^8$	$2.7\times10^9\pm1.2\times10^8$
	3rd day of incubation	$1.1\times10^9\pm9.2\times10^7$	$3.3\times10^8\pm1.1\times10^7$	$9.8\times10^8\pm7.2\times10^7$	$5.9\times10^8\pm2.8\times10^7$	$8.4\times10^8\pm4.6\times10^7$	$2.2\times10^9\pm1.0\times10^8$
26	Before incubation	$6.0\times10^8\pm5.5\times10^7$	$6.0\times10^8\pm5.5\times10^7$	$6.0\times10^8\pm5.5\times10^7$	$6.0\times10^8\pm5.5\times10^7$	$6.0\times10^8\pm5.5\times10^7$	$6.0\times10^8\pm5.5\times10^7$
	3rd day of incubation	$1.1\times10^9\pm6.3\times10^8$	$1.2\times10^9\pm2.0\times10^8$	$1.1\times10^9\pm1.0\times10^8$	$1.3\times10^9\pm9.8\times10^7$	$1.1\times10^9\pm7.9\times10^7$	$1.1\times10^9\pm1.1\times10^8$

The results (Table VI) showed that all investigated isolates No. 14, 15, 21, 26 had the ability to biodegrade at least three mycotoxins used in the work. Perhaps this is due to the presence of enzyme strains in the culture fluids that carry out the biodegradation of mycotoxins. The results are consistent with data from other researchers. Fuchs et al. [25] previously examined the ability of the bacterial strain BBSH +797 isolated from the cattle rumen to biotransform T-2 mycotoxin, HT-2 toxin, T-2 triol, T-2 tetraol, skirpentriol, and diacetoxiskircenol. Strain BBSH +797 was described by researchers as a gram-positive, non-spore-forming, strictly anaerobic bacillus of 0.2-0.4 x 1-1.5 microns in size. As a result of the study, it was demonstrated that all the studied mycotoxins underwent active biotransformation with the participation of this strain. It was shown that T-2 toxin was partially hydrolyzed to NT-2 toxin, skirpentriol was transformed to nontoxic diepoxiscirpentriol, HT-2 toxin was degraded to its di-epoxy-HT-2 toxin, T-2 tetraol to di-epoxy T-2 tetraol.

TABLE VI. LEVEL OF MYCOTOXINS' BIODEGRADATION WITH RUMEN MICROORGANISMS ISOLATES, % (M±M, N=3)

Isolate collection number	Aflatoxin	Ochratoxin A	T-2 mycotoxin	ZEN	DON
14	75.1±3.9	absent	3.3±0.14	absent	absent
15	47.8±2.6	5.8±0.38	19.9±1.3	absent	5.1±0.19
21	61.2±3.4	absent	10.8 ± 0.47	14.7 ± 1.42	absent
26	46.1±2.9	8.3±0.43	87.5±5.1	50.9 ± 2.7	13.4±1.04

We observed the highest level of biodegradation for aflatoxinsm, which reached 75.1±3.9%. This is probably due to the fact that AFL molecules have a flat conjugated system of bonds between electrons, capable of binding to cell walls due to dispersion π-π interactions. Similar experiments were previously carried out by Peltonen et al. [26]. Researchers studied the biodegradability of aflatoxin B1 (AFL B1) by strains of lactic acid bacteria. The most active degradation ability of this mycotoxin was detected in a strain of the microorganism *Lactobacillus rhamnosus*. So, the decomposition of AFL B1 was 80% within an hour after the

introduction of bacterial cells into the culture medium. Later, by a team of authors [27], it was shown that the strain of the *Mycobacterium fluoranthenivorans* DSM44556T bacterium had the ability to decompose aflatoxin B1. Moreover, 36 hours after the start of the experiment, a decrease in the concentration of AFL B1 was observed by 70-80% compared to its initial amount, and after 72 hours – by 100%. It was also demonstrated [27] that the bacterial strain *Rhodococcus erythropolis* showed an active ability to degrade AFL B1. So, 48 hours after the introduction of the cells of the microorganism *R. erythropolis* into a liquid medium containing AFL B1, the concentration of this mycotoxin was 17% of the initial amount, after 72 hours it was only 3-6%.

IV. Conclusion

Our experiments led us to the conclusion that the isolates of microorganisms isolated from the reindeer rumen are capable of synthesizing cellulases and enzymes that biodegrade mycotoxins, which probably allows them to gain a competitive advantage in the rumen of a deer that consumes diets with an abundance of indigestible fiber and secondary metabolites with toxic properties.

Due to the fact that the basis of the modern concept of combating pathogens and their toxins is the optimization of microecological niches of agroecosystems, which should take into account the principle of self-regulation, the obtained result in the study of microorganisms with high biological activity is of particular importance. These strains are of undoubted interest for livestock (including dairy cattle and reindeer husbandry) with the aim of using them as environmentally friendly and safe probiotic feed additives.

References

[1] A.A.S. Araújo, M.G.D. de Melo, T.K. Rabelo, P.S. Nunes, S.L. Santos, M.R. Serafini, M.R.V. Santos, L.J. Quintans-Júnior and D.P. Gelain, "Review of the biological properties and toxicity of usnic acid," Natural Product Research, Vol. 29, No. 23, pp. 2167-2180, 2015. https://doi.org/10.1080/14786419.2015.1007455

[2] T.Yu. Tolpysheva, Biotic relationships of lichens in forest and bog ecosystems: Authors Abstract of Candidate of Science (PhD) Dissertation (Agricultural Science), Petrozavodsk, 2005. (in russ.)

[3] T.Yu. Tolpysheva, "Mycotoxins and usnic acid and their distribution in lichens belonging to the genera Cetraria, Flavocetraria, and Cladonia," Vestnik Moskovskogo universiteta. Seriya 16: Biologiya (Herald of Moscow University. Series 16. Biology), No. 3, pp. 37-41, 2014. (in russ.)

[4] A.A. Burkin and G.P. Kononenko, "Peculiarities of mycotoxin accumulation in lichens," Prikladnaya biohimiya i mikrobiologiya (Applied Biochemistry and Microbiology), Vol. 49, No. 5, pp. 521-528, 2013. (in russ.)

[5] A.A. Burkin and G.P. Kononenko, "Secondary fungal metabolites (mycotoxins) in lichens of different taxonomic groups," Izvestiya Rossijskoj akademii nauk. Seriya biologicheskaya (Proceedings of the Russian Academy of Sciences. Biological Series), Vol. 41, No. 3, pp. 216-222, 2014. (in russ.)

[6] E.A. Yildirim, L.A. Ilyina, K.A. Layshev, V.A. Filippova, A.V. Dubrovin, T.P. Dunyashev, G.Yu. Laptev, I.N. Nikonov, A.A. Yuzhakov, T.M. Romanenko and Yu.P. Vylko, "Mycotoxins diffusion in feeds of summer pasturing ration of Rangifer tarandus in arctic zones of Russia," Selskohozyajstvennaya biologiya (Agricultural Biology), Vol. 53, No. 4, pp. 779-786, 2018. (in russ.)

[7] T. Zhang, Z.Q. Shi, L.B. Hu and L.G. Cheng, "Antifungal compounds from Bacillus subtilis B-FS06 inhibiting the growth of Aspergillus flavus," World Journal of Microbiology and Biotechnology, Vol. 24, pp. 783-788, 2008.

[8] K.M. Cho, R.K. Math, S.Y. Hong, Sh.Md.A. Islam, D.K. Mandanna, J.J. Cho, M.G. Yun, J.M. Kim and H.D. Yun, "Iturin produced by Bacillus pumilus HY1 from Korean soybean sauce (kanjang) inhibits growth of aflatoxin producing fungi," Food Control, Vol. 20, No. 4, pp. 402-406, 2009. https://doi.org/10.1016/j.foodcont.2008.07.010

[9] K.R.N. Reddy, C.S. Reddy and K. Muralidharan, "Potential of botanicals and biocontrol agents on growth and aflatoxin production by Aspergillus flavus infecting rice grains," Food Control, Vol. 20, No. 2, pp. 173-178, 2009. https://doi.org/10.1016/j.foodcont.2008.03.009

[10] G. Schatzmayr, F. Zehner, M. Tãubel, D. Schatzmayr, A. Klimitsch, An. P. Loibner and E.M. Binder, "Microbiologicals for deactivating mycotoxins," Molecular Nutrition and Food Research, Vol. 50, No. 6, pp. 543-551, 2006.

[11] L. Abrunhosa, A. Inês, A.I. Rodrigues, A. Guimarães, V.L. Pereira, P. Parpot, A. Mendes-Faia and A. Venâncio "Biodegradation of ochratoxin A by Pediococcus parvulus isolated from Douro wines," International Journal of Food Microbiology, Vol. 188, pp. 45–52, 2014. https://doi.org/10.1016/j.ijfoodmicro.2014.07.019

[12] R.T. Palo "Usnic acid, a secondary metabolite of lichens and its effect on in vitro digestibility in reindeer," Rangifer, Vol. 13, No. 1, pp. 39-43, 1993. https://doi.org/10.7557/2.13.1.1071

[13] M.A. Sundset, J.E. Edwards, Y.F. Cheng, R.S. Senosiain, M.N. Fraile, K.S. Northwood, K.E. Prasteng, T. Glad, S.D. Mathiesen and A.G. Wright, "Rumen microbial diversity in Svalbard reindeer, with particular emphasis on methanogenic archaea," FEMS Microbiology Ecology, Vol. 70, No. 3, pp. 553-562, 2009. https://doi.org/10.1111/j.1574-6941.2009.00750.x

[14] O.A. Luzin and N.F. Salakhutdinov, "Biological activity of usnic acid and its derivatives: Part 2. effect on higher organisms. Molecular and physicochemical aspects," Bioorganicheskaya himiya (Russian Journal of Bioorganic Chemistry), Vol. 42, No. 3, pp. 249-268, 2016. (in russ.)

[15] E.K. Borozdin, V.A. Zabrodin and P.N. Vostryakov, Reindeer herding. Moscow: Kolos, 1979.

[16] E.Z. Tepper, V.K. Shil'nikova and G.I. Pereverzeva, Workshop on microbiology. Moscow: Kolos, 1993.

[17] T.K. Chyurlis, On the methodology for determining the activity of cellulose-breaking microflora of the pancreas in cattle. Feeding farm animals. Moscow: Agricultural Publishing House, 1958.

[18] B.V. Cockroaches, Research methods of the microflora of the digestive tract of farm animals and birds. Moscow: Scientific World, 2006.

[19] G.F. Lakin, Biometrics. Moscow: Higher school, 1990.

[20] M.J. Wolin, I.W. McDonald and A.C.I. Warner, Interactions between the bacterial species in the rumen. Australia: University New England Publishing Unit, 1975.

[21] S. Moraïs and I. Mizrahi, "Islands in the stream: from individual to communal fiber degradation in the rumen ecosystem," FEMS Microbiology Reviews, Vol. 43, No 4, pp. 362-379, 2019. https://doi.org/10.1093/femsre/fuz007

[22] M. Ivan, H.V. Petit, J. Chiquette and A.D.Br. Wright, "Rumen fermentation and microbial population in lactating dairy cows receiving diets containing oilseeds rich in C-18 fatty acids," The British Journal of Nutrition, Vol. 31, pp. 1-8, 2012.

[23] B. Michalet-Doreau, I. Fernandez and G. Fonty, "A comparison of enzymatic and molecular approaches to characterize the cellulolytic microbial ecosystems of the rumen and the cecum," Journal of Animal Science, Vol. 80, No. 3, pp. 790-796, 2002. https://doi.org/10.2527/2002.803790x

[24] P.J. Weimer, "Why Don't Ruminal Bacteria Digest Cellulose Faster?," Journal of Dairy Science, Vol. 79, pp. 1496-1502, 1996.

[25] E. Fuchs, E.M. Binder, D. Heidler and R. Krska, "Structural characterization of metabolites after the microbial degradation of type A trichothecenes by the bacterial strain BBSH 797," Food Additives and Contaminants, Vol. 19, No. 4, pp. 379-386, 2002. https://doi.org/10.1080/02652030110091154

[26] K.D. Peltonen, H.S. El-Nezami, S.J. Salminen and J.T. Ahokas, "Binding of aflatoxin B1 by probiotic bacteria," Journal of the Science of Food and Agriculture, Vol. 80, No. 13, pp. 1942–1945, 2000.

[27] Q.K. Wu, A. Jezkova, Z. Yuan, L. Pavlikova, V. Dohnal and K. Kuca, "Biological degradation of aflatoxins," Drug metabolism review, Vol. 41, No. 1, pp. 1-7, 2009. https://doi.org/10.1080/03602530802563850