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TOXIN-PRODUCING FUNGI OF THE GENUS *Penicillium* IN COARSE FODDERS

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Abstract

The search for toxin-producing microscopic fungi, affecting plant and animals products, food and feed continues to be relevant in scientific research. A long-term study of this problem has shown that the threat of the occurrence of toxicoses of humans and animals is associated mainly with micromycetes of genus *Fusarium*, *Aspergillus* and *Penicillium* (CAST, 1989). In coarse feed, which form the basis of the ration of ruminants, these fungi have a leading position. Among *Fusarium* fungi the highly toxic species *F. sporotrichioides* dominates in hay and straw (E.A. Piryazeva et al., 2016), and 7 species of *Aspergillus* are capable to contaminate that with CPA, STE and MPA (G.P. Kononenko et al., 2017). The purpose of this work was to elucidate the toxin-forming potential of 11 species of the genus *Penicillium* fungi which prevail in mycobiota of coarse feed. The strains were cultured for 7 days at 25 °C on a panel including Czapek Dox agar (CDA), wort agar (WA), Czapek Yeast Autolysate Agar (CYA), yeast extract sucrose agar (YES) and moistened rice grain (RG). Further, in the extracts, the amounts of ochratoxin A (OA), citrinin (CIT), mycophenolic acid (MPA), PR-toxin (PR), cyclopiazonic acid (CPA), emodin (EMO) and ergot alkaloids (EA) were determined by indirect competitive enzyme-linked immunosorbent assay (ELISA) using certified commercial and research test systems. On the basis of obtained results, the species *P. aurantiovirens*, *P. palitans*, as well as *P. martensii* and *P. meleagrinum* are classified as non-producing. Among the representatives of *P. cyclopium*, we found weak producers of MPA and CIT, as well as isolates that do not form any of the analyzed mycotoxins. High accumulation (10 μg/g and more) is revealed in *P. brevicompactum*, *P. stoloniferum* (MPA), *P. roqueforti* (PR + MPA), *P. chrysogenum* (PR) and average level (1-10 μg/g) is characteristic of *P. urticae* (CPA) and *P. expansum* (CIT). Isolates lacking production capacity were found only among *P. chrysogenum* and *P. expansum*, toxin production by other species was stable. In the representatives of *P. roqueforti*, producing jointly PR and MPA, the amount of MPA was, as a rule, less than PR. EMO, OA and EA in the metabolites of fungi were not found. In this paper it was shown for the first time that the species *P. expansum*, *P. brevicompactum*, *P. stoloniferum*, *P. roqueforti*, *P. chrysogenum* and *P. urticae* from a typical complex of fungi of this genus can be related to extensive and intensive contamination of coarse fodder by CIT, MPA, PR and CPA. It has been established that the use of a growth media panel for testing the toxicity of *Penicillium* fungi is an indispensable technique for an exhaustive assessment of their potential, and, in addition to agar media, it is expedient to use substrates of plant origin. Virtually all producing species had the highest intensity of toxin accumulation on RG, and for *P. chrysogenum*, PR production could be detected only on this substrate. Specific features of the profile of toxic metabolites in isolates belonging to one species are discussed, as well as the problem of the possible contribution of other species of *Penicillium* to the contamination of coarse fodder by mycotoxins.

Keywords: mycobiota, feeds, *Penicillium* fungi, mycotoxins

The search for mycotoxin producers among microscopic fungi which affect food and forage crops, animal products, food stuffs and feeds is still topical in scientific researches [1, 2]. The greatest threat of toxicoses occurrence is related to the micromycetes of the *Fusarium, Aspergillus* and *Penicillium* genera [3, 4]. In roughage fodder which forms the basis of the ration of ruminants the fungi of these taxa have a leading position, however, the diversity of the botanical

composition of herbages and differences in storage conditions after the harvesting imply the conduction of regional surveys with the evaluation of the individual toxin-forming ability of the species being a part of the mycobiota.

The world's data on this issue are very poor. In Russia, the data on mycological analysis of the samples from production batches of hay and straw harvested in livestock farms of the Bryansk, Moscow and Chelyabinsk regions and subsequent testing of taxonomically classified isolates show that among *Fusarium* fungi the highly toxic *F. sporotrichioides* species dominates [5], and the complex consisting of 7 *Aspergillus* species may be related to the contamination of the fodder with cyclopiazonic acid, sterigmatocystin and mycophenolic acid [6]. According to the data of mycological examination of the production batches of hay and straw harvested in the Moscow and Belgorod regions in 2011 and 2013, more than half of the samples were contaminated with *Penicillium* fungi which belong to 28 species from five sections of this genus, 11 species of were being found with the frequency of 3.8-30.8% (7).

In this paper, for the first time, we showed that the *P. brevicompactum, P. stoloniferum*, *P. roqueforti*, *P. chrysogenum*, *P. urticae*, and *P. expansum* species belonging to *Penicillium* genus may be related to the extensive and intensive contamination of roughage fodder with mycophenolic acid, PR toxin, cyclopiazonic acid and citrinin.

Our objective was to investigate the toxin-producing ability of *Penicillium* species predominating in roughage fodder mycobiota in the experimental conditions ensuring the most complete performance of biosynthetic capabilities of these fungi.

Techniques. We studied 55 *Penicillium* isolates of *P. aurantio-virens* Biourge, *P. brevi-compactum* Dierckx, *P. chrysogenum* Thom, *P. cyclopium* Westling, *P. expansum* Link, *P. martensii* Biourge, *P. notatum* Westling, *P. palitans* Westling, *P. roqueforti* Thom, *P. stoloniferum* Thom, *P. urticae* Bainier species, which have been isolated from the roughage fodder harvested in the livestock farms of the Bryansk and Moscow regions [7], as well as 134 strains of the same species from the Collection of the Feed Mycotoxicology and Sanitation Laboratory of the All-Russian Research Institute of Veterinary Sanitation, Hygiene and Ecology. The identification of the species was based on the cultural and morphological properties according to the taxonomic system [8] with the use of species epithets from the up-to-date nomenclature database of the MycoBank (http://www.mycobank.org/) and with the following changes: *P. aurantio-virens* (=*P. aurantiovirens*), *P. brevi-compactum* (=*P. brevicompactum*) и *P. notatum* (=*P. meleagrinum* Biourge) [9].

The evaluation of toxin formation included the preparation of the inoculum and substrate, inoculation, culture, extraction, and analysis of mycotoxins. The 10-day-old cultures on Czapek-Dox agar (CDA, HiMedia Laboratories Pvt., Ltd., India) were used for inoculums. Approximately equal size pieces taken from the agar surface with the mycological hook was placed in 3 replicates on the grain substrate or on solid culture media in 15 ml glass bottles with the bottom diameter of about 18 mm. The sterile crushed rice (1 g) previously moistened with 1 ml of water was a grain substrate (RG). The CDA, wort agar (WA, Liofilchem, Italy), as well as Czapek Yeast Autolysate (CYA) and yeast extract sucrose agar (YES) [10] prepared of the commercial components (HiMedia Laboratories Pvt., Ltd, India) were used as growth media (1.5 ml each).

After adding of the inoculum, the bottles were closed with cotton-gauze plugs, which were wrapped with a laboratory film (Parafilm "M"® PM-996, Pechiney Plastic Packaging, USA). The culturing was performed in the dark for 7 days at 25 °C. Then the mixture of acetonitrile and water (84:16 v/v) was add-

ed to each bottle and intensively shaken at the beginning and in the end of the 14-hour stationary extraction. The content of ochratoxin A (OA), citrinin (CIT), mycophenolic acid (MPA), cyclopiazonic acid (CPA), PR toxin (PR), emodin (EMO) and ergoalkaloids (EA) in the extracts was determined by the enzyme-linked immunosorbent assay (ELISA) using certified test systems [8], the lower limits of detection corresponded to 85% antibody binding.

The data were processed using Microsoft Excel 2013 for descriptive statistics, the results were expressed as the absolute content of mycotoxin or as the arithmetic means of the obtained values (*M*) with a standard error of the mean (±SEM).

Results. In recent years, a polyphase approach has been widely used in the systematics of microscopic fungi. Along with the morpho-cultural and molecular-genetic characterization, this approach takes account of biochemical properties. There is a quite large database on the *Penicillium* fungi profile of secondary metabolites jointly called "extrolites" which include physiologically active substances and mycotoxins [12-15]. However, detecting of the substances in a semi-quantitative assessment mode visually or using instrumental physicochemical methods does not make it possible to conclude about the intensity of their biosynthesis and about the completeness of the obtained information. In this regard, at the preparatory stage of the work, we quantified toxin formation in a standard experiment on WA with collection cultures of 11 the most abundant species of roughage fodder mycobiota from *Penicillium* genus isolated from different subjects (Table 1).

1. Toxin formation in the collection strains of 11 species of the *Penicillium* fungi (WA, 23 °C, 7 days)

Penicillium species (*n*)	Mycotoxin	n^+ (mycotoxin content min-max, μg per 1 g of the substrate)
P. aurantiovirens (12)	CIT	4 (0.1-0.3)
P. brevicompactum (7)	CIT	7 (40-440)
P. chrysogenum (8)	PR	4 (0.8-40)
	EMO	2 (0.1; 0.6)
P. cyclopium (24)	PR	1 (0.7)
	CIT	3 (30, 40, 50)
P. expansum (3)	CIT	2 (13, 16)
P. martensii (24)	CPA	1 (0.6)
P. meleagrinum (3)	–	–
P. palitans (11)	–	–
P. roqueforti (16)	PR + CIT	7 (35-135)+(0.2-25)
	PR	1 (20)
P. stoloniferum (3)	CIT	3 (2, 22, 76)
P. urticae (23)	CPA	17 (0.2-4)
	CIT	1 (40)

N o t e. WA — wort agar; *n* — number of investigated strains, n^+ — number of producers; CIT — citrinin, MPA — mycophenolic acid, CPA — cyclopiazonic acid, EMO — emodin, PR — PR toxin; "–" means that no producers were found.

In all species except *P. meleagrinum* and *P. palitans*, the producers of CIT, MPA, CPA and EMO have been found. However, only in *P. brevicompactum* and *P. stoloniferum* showed a complete realization of the potential; in other species, the strains which are unable for the production were found: CIT was found only in four of 12 *P. aurantiovirens* strains, and CPA only in one of 24 *P. martensii* strains (see Table. 1). The producers of different toxins were identified among *P. chrysogenum, P. cyclopium* and *P. urticae*; most of the *P. roqueforti* strains synthesized both PR and MPA, and one strain only PR. This indicates the existence in these species of intraspecific chemotypes or subspecies with the specific profile of mycotoxins [13] or of atypical forms with specific features of metabolic ways.

In order to assess the toxin formation potential of fungi isolated from roughage fodder, groups were formed, 5 cultures of each of 11 species per group,

and a panel of 5 culture media was composed. Given our previous data on metabolic response of fungi isolated from the same subjects, we used WA and RG taken for fungi of the *Fusarium* genus [5], and CDA that we earlier applied for testing *Aspergillus* [6]. Among the solid media recommended for growth and identification of *Penicillium* [10, 12-15], we chose CYA and YES on which two *P. stoloniferum* strains from the Collection showed more accumulation of MPA (38±8 and 73±8 μg/g, 230±36 and 305±22 μg/g, respectively) than on the agar with the malt extract (MEA) (2.0±0.4 and 22±3 μg/g).

In these experiments, we did not find any toxin formation in *P. aurantiovirens* ($n = 5$) and *P. palitans* ($n = 5$) isolates. This result was expected because the collection strains of the first type had extremely weak production of CIT, and the strains of the second type did not produced CIT at all (see Table 1). Nevertheless, it is important to note that there are many papers on taxonomy which report the ability of *P. palitans* to synthesize CPA [15], therefore, we should admit the probability of some uncertainty in the identification of this species basing on morphological properties. In *P. martensii* ($n = 5$) and *P. meleagrinum* ($n = 5$) we could determine CIT only sporadically on WA and RG in the amounts less than 1 μg/g, and we have not found CPA, although it has been found in small amount in one of 24 collection strains of *P. martensii* (see Table. 1). In *P. cyclopium*, the toxin formation was also weakly expressed and multivariate. Particularly, on CDA, WA, and RG one isolate produced small amounts of MPA (0.3-1.0 μg/g), while other isolate produced CIT (0.03-0.8 μg/g), moreover, according to data of chemotaxonomic researches, this species is not capable of biosynthesis of the analyzed mycotoxins at all [15]. The results indicated that the contribution of these five species (*P. aurantiovirens*, *P. cyclopium*, *P. martensii*, *P. meleagrinum*, and *P. palitans*) to contamination of roughage fodder with the sanitarily significant mycotoxins is inconsiderable.

In the rest six species (*P. brevicompactum*, *P. stoloniferum*, *P. roqueforti*, *P. chrysogenum*, *P. urticae*, and *P. expansum*), high production of MPA and PR (more than 10 μg/g) and medium production of CPA and CIT (up to 10 μg/g) has been confirmed (Table. 2). As expected, *P. brevicompactum* and *P. stoloniferum* were characterized by stable biosynthesis of MPA; no other toxins from the list of analyzed ones have been found. The significant intensity of accumulation of this toxin allowed us to attribute these isolates to highly active producers and highly probable sources of feed contamination [16].

It has been confirmed that all isolates of *P. roqueforti* from roughage fodder are able to produce PR in combination with MPA (see Table 2), which is deemed a hallmark of the *P. roqueforti* var. *roqueforti* subspecies [13]. The reaction of the tested cultures to the growth medium was very peculiar. On the depleted CDA substrate, the intensity of PR accumulation was significantly higher than that on WA, although it was previously reported that the isolates of this species from silage feeds had almost no differences in toxin formation on CDA and WA, and MPA were also found in much smaller amounts than PR [17].

The reason of this discrepancy could well be the using in the said work of the substrate prepared of beer wort in laboratory conditions, and the observed sharp increase in biosynthesis of PR on the analogue of a commercial standardized medium undoubtedly deserves attention. The metabolic response of the *P. roqueforti* isolates to CYA and YES generally was weakly expressed and similar in terms of the proportion of PR and MPA amounts. The differences between the strains have been noted only on CYA, and on the other solid media were insignificant. Nevertheless, recently, when testing for YES with visual TLC detection, the strains of *P. roqueforti* from different cheese varieties, which jointly produce PR and MPA, showed significant differences in the intensity of PR

accumulation and minor differences in MPA [18]. It is quite possible that the morphologically identical cultures from different subjects have genetically determined features and, as a consequence, show dissimilar metabolic responses to changes in growth conditions.

2. Toxin formation in isolates of *Penicillium* fungi species from roughage fodder on solid media and on the rice grain substrate (25 °C, 7 days)

Mycotoxin	Isolate No.	Mycotoxin concentration, μg/g substrate (M±SEM)				
		CDA	WA	CYA	YES	RG
		P. brevicompactum (n^+/n = 6/6)				
MPA	40/2	6.0±0.9	6,0±1,1	76±15	110±20	124±25
	181/1	9.0±1.8	17±3	34±7	174±35	302±70
	254/1	8.0±1.6	15±3	28±6	100±18	169±32
	4/3	–	128±30	126±32	120±18	722±101
	340/1	–	81±10	144±25	192±25	1117±91
	16/1	–	53±8	19±4	72±8	792±19
		P. chrysogenum (n^+/n = 4/5)				
PR	172/1	0	0	0	0	14±3
	373/1	0	0	0	0	13±2
	592/1	0	0	0	0	5,7±1,3
	639/5	0	0	0	0	2,0±0,4
		P. expansum (n^+/n = 1/5)				
CIT	88/4	5.0±0.8	3.0±0.5	7.0±1.7	3.3±0.6	11±2)
		P. roqueforti (n^+/n = 5/5)				
PR + MPA (the values are separated with slash)	88/2	50±9/ 1±0	2.8±0.5/ 3.0±0.5	4.3±0.7/ 3.1±0.6	5.7±1.8/ 0.13±0.02	150±24/ 63±13
	118/1	45±8/ 1±0	0.9±0.2/ 2.5±0.5	1.7±0.3/ 0.9±0.2	2.0±0.3/ 0.13±0.02	95±14/ 48±8
	648/5	48±9/ 0.2±0.0	10±2/ 3.3±0.6	6.7±1.0/ 4.7±0.9	3.0±0.4/ 0.07±0.01	780±72/ 67±14
	393/1	29±)/ 0.3±0.1	2.1±0.4/ 5.3±1.0	50±9/ 8.7±1.5	4.7±0.8/ 3.7±0.6	329±43/ 98±17
	557/1	22±3/ 0	4.7±0.6/ 2.7±0.4	34±6/ 3.4±0.7	2.3±0.4/ 0.1±0.02	224±38/ 55±8
		P. stoloniferum (n^+/n = 5/5)				
MPA	631/4	0	11±2	43±5	64±13	100±20
	631/3	0	14±4	41±10	91±10	175±39
	317/4	10±2	193±39	115±22	151±30	1330±250
	602/1	5.7±1.7	24±5	40±6	272±54	332±60
	602/3	0	9±2	23±4	110±20	145±22
		P. urticae (n^+/n = 5/5)				
CPA	349/1	1±0	0.1±0	5.7±0.6	2.3±0.5	4.0±0.8
	584/4	2.0±0.2	0.1±0	12±2	1±0	10±2
	201/1	2±0	0.1±0	7±1	3.3±0.6	12±1
	216/5	0	0	0.43±0.07	0.23±0.05	0.37±0.07
	434/4	2±0	0.33±0.06	5.3±0.6	5±0	6.7±1.3

N o t e. CDA — Czapek-Dox agar, WA — wort agar; CYA — Czapek yeast autolysate agar; YES — yeast extract sucrose agar, RG — rice grain; n — number of investigated strains. n^+ — number of producers; CIT — citrinin, MPA — mycophenolic acid, CPA — cyclopiazonic acid, PR — PR toxin; "–" means that the mycotoxin detection was not performed.

All representatives of *P. urticae* demonstrated the ability to synthesize CPA although with the accumulation of the amount less than 10 μg/g. This is consistent with the data of TLC testing (synthetic medium, 28 °C, 8 days) of 13 isolates of the same species (isolated from dried beans and macaroni products), which also turned out to be the producers of this toxin [19]. In accordance with the new taxonomic approach, *P. urticae* is deemed the synonymous of *P. griseofulvum* Dierckx [14], and CPA is deemed one of the specific metabolic markers [15].

In *P. chrysogenum* and *P. expansum* species, the full realization of the potential has not been noted. All members of *P. chrysogenum*, except for one, produced PR in a wide quantitative range and only on the grain substrate (see Table 2). The absence of toxins formation on the wide panel of solid media was unexpected, because in the aforementioned work [12] it was reported that, on CYA and YES, 50-80% isolates of this species of 87 investigated ones are capable of PR biosynthesis, and most strains from the All-Russian Collection of Mi-

croorganisms of the Skryabin Institute of Biochemistry and Physiology of Microorganisms RAS formed this toxin although in small amounts [17].

Only one of the five *P. expansum* strains synthesized CIT on all media (see Table 2); an analogous partial production was noted when testing the collection strains on WA (see Table 1). Apparently, "zero" chemotypes of this fungus species are found in fodder subjects quite often. However, it should be noted that when TLC screening (CYA, YES) of *P. expansum* from different sources, CIT was detected in almost all strains of different origin (95-99%, $n = 91$) [9].

The obtained results show that the choice of growth media panel is necessary for a comprehensive evaluation of toxin formation in microscopic fungi, and using of standardized commercial media for investigating biochemical processes is rather necessary than preferable. A particular attention should be paid to the qualitative differences in the fungal response when changing the growth medium (for example, as in case of *P. roqueforti* and *P. chrysogenum*). In recent years, the structure of the genome regions which are responsible for the synthesis of toxic metabolites has been investigated in detail for these species, and the important role of the extra-cluster regulators, the activity of which is determined by growth conditions, in particular, by the properties of growth media, has been shown [21-23]. In our experiments with the fungi of *Penicillium* genus, more active accumulation of MPA, PR, CPA, and CIT occurred on the rice grain substrate. Only on this substrate, four of five *P. chrysogenum* strains produced PR which is considered one of its chemotaxonomic markers. Expanding the range of natural substrates will make it possible in the future to create the analogous formulations with controlled composition which will ensure the maximum realization of the metabolic potential of fungi.

The data set forth in the work testify that the *P. brevicompactum*, *P. stoloniferum*, *P. roqueforti*, *P. chrysogenum*, *P. urticae*, and *P. expansum* species can be related to an extensive and intensive contamination of roughage fodder with MPA, PR, CPA and CIT [24]. However, it is known that the fungi of this genus, when living in anthropogenically disturbed and extreme conditions, can form morphologically modified phenotypes of an adaptive nature [25]. Thus, when testing some strains of *P. cyclopium* and *P. urticae* on WA, we found the highly active producers of a metabolite which is atypical, the MPA (see Table 1). These producers were isolated from caked layers of crumbled feed affected by long-term self-heating. In the described case, it is quite possible that the species attribution based on micro- and macromorphological characters will be wrong.

Moreover, the participation of other representatives of the *Penicillium* genus can hardly be completely excluded, because the species which have been selected for the assessment as the most represented in the mycobiota comprise only less than half of their total number. In particular, for the isolate attributed to the *P. steckii* Zaleski (=*P. steckii* K.M. Zalesski) [9], (=*P. citrinum* Thom) [26] species rarely occurred in such feed the active production of CIT has been shown (our own unpublished data).

The mycotoxins frequently occurred in roughage feed, i.e. OA, EMO, and EA, have not been found in the tested isolates. Nevertheless, among the rarely occurred species, we have identified P. viridicatum [7] which includs, as it has been proved, several lines (in the status of a species or subspecies) with distinct differences in toxin formation. Some of these are P. verrucosum and P. nordicum which produce OA [27]. The frequent detection and high content of EMO in feed still have no satisfying explanation in the mycological aspect. The arguments for attribution of EMO to the associated metabolites of higher plants and of specific endophytic fungi prevail [28], although several species of the *Penicillium* genus, in particular *P. islandicum*, *P. brunneum*, *P. janthinellum*, and

P. herquei, have been attributed to the producers of this metabolite [29]. The sources of extensive contamination of roughage feed with EA also remain unknown. Among the taxa classified as non-traditional sources of peptide EA, only the imperfect fungi of the *Aspergillus*, *Botrytis*, *Curvularia* and *Geotrichum* genera, lower fungi (*Cinnighamella blakesleana, Mucor hiemalis* and *Rhyzopus* spp.), as well as the grass endophytes belonging to ascomycetes (*Balansia* spp., *Epichloe typhina*, *Hypomyces aurantius*, *Sepedonium* sp.) are named [30]. However, recently in an isolate from mixed cereal-bean hay harvested in 2005 in the Perm Territory, which has been identified as *P. palitans* according to cultural properties, the active accumulation of these metabolites (2.7±0.2 μg/g) on WA has been detected (our own unpublished data).

It cannot be ruled out that the *Penicillium* fungi are related to the contamination of such feeds also with other toxins from the list of frequently occurred ones. Thus, in the separate experiment, alternariol was found in small amounts in the isolate of *P. steckii* and two isolates of *P. urticae* grown on MEA, CYA, and YES (our own unpublished data). It was also previously reported about the ability of *P. coprophilum* to synthesize this toxin [15].

The important outcome of the presented work is the understanding that the improvement of the methodology of evaluating the toxins formation in microscopic fungi must be continued. For many decades, the science has accumulated the rich experience in the study of toxins formation in microscopic fungi, but the information about the potential of the populations which vital activity is related to agricultural plants is still very poor, and the available experimental data are often the subject of incorrect interpretations and conclusions. One of the main reasons of it is the underestimation of the complexity of this problem and the absence of a generally accepted methodology of conducting the research works. Toxin-producing ability should be evaluated under unified conditions to make the results comparable [31]. In recent years, the simple technology of short-term growing on nutrient substrates with screening analysis ensuring wide diapason of measurement is gaining the increasing recognition. The distinction level of 10 μg/g and relevant terms, i.e. “weak producers” with less accumulation and “highly active producers” with accumulation above the said threshold value, have already been proposed to differentiate isolates for their intensity of toxin formation [32]. The ratio of the producers number to the total number of investigated strains denoted as n^+/n (in percent) is usually called the toxin formation potential in the analyzed aggregate of isolates, and the accumulation diapason denoted as min-medium-max (μg/g) is called the toxin formation intensity. The term “toxin formation pattern” may be applied to identify qualitative differences in metabolic profiles within a set of producers.

The *Penicillium* fungi prevailing in the composition of roughage feed mycobiota produce the mycotoxins and thus can suppress microbial activity in ruminants, which leads to serious violations of the rumen function and provoke animal intoxication [33]. In addition, microscopic fungi along with bacteria and protozoa actively participate in assimilation of polysaccharides of the fodder plant cell walls. Recently it was shown that fungi of the *Aspergillus* and *Penicillium* genera, one of which is *P. brevicompactum* [34], dominate in the partially digested cellulose in cow abomasum. The consequences of long-term anaerobic habitation of toxigenic fungi in the digestive tract of such animals at each of the four successive stages may be very dangerous and need to be studied in details.

Thus, six species of *Penicillium* fungi (*P. brevicompactum*, *P. stoloniferum*, *P. roqueforti*, *P. chrysogenum*, *P. urticae* and *P. expansum*), represented in the composition of the roughage feed mycobiota are capable of active producing a complex of mycotoxins and may be related to massive and intensive contamina-

tion by citrinin, mycophenolic acid, PR-toxin and cyclopiazonic acid. It has been established that the choice of the panel of growth media for testing toxin formation in *Penicillium* fungi is necessary to comprehensively evaluate their potential. Along with solid media, substrates of plant origin should be used. Knowing of the biosynthetic capabilities of microscopic fungi is important not only for solving such global practical problems as animal safety and safe products of agriculture, but also for the further development of fundamental molecular genetics and evolutionary concepts about the vital activity of these organisms.

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