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Micromycetes of Damaged Documents and Air Environment of Book Depositories as Potential Cellulase Producers

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Abstract

The destruction of indigestible cellulose-containing substrates makes it possible to utilize waste from many industries and obtain a variety of enriched feed products. In this regard, the search for organisms capable of splitting celluloses into regular oligosaccharides suitable for further processing is an urgent task of biotechnology. The purpose of the study was to assess the species diversity of micromycetes of library documents and the air of book storages and to determine their prospects as potential cellulase producers. The number of microorganisms on the surface of the documents was determined by the method of prints using wet sterile filter paper disks or cotton swabs. Air samples using the MAS-100 Eco aspirator. Micromycetes were isolated and identified on the basis of cultural and morphological features using light microscopy. Based on the results of a qualitative assessment, the activity of the cellulase complex was determined. The following strains were isolated from the surface of documents: Penicillium canescens, Penicillium aurantiogriseum, Penicillium simplicissimum, Aspergillus sydowi, Talaromyces funiculosus, Chaetomium globosum, Cladosporium cladosporioides.

Discipline: Microbiology.

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1 Introduction

Micromycetes, known as biodegraders of various materials, are found in all cultural heritage sites in countries with a wide variety of climatic conditions. Numerous studies devoted to the isolation and study of microorganisms that damage historical objects, including book monuments, show a significant variety of isolated microorganisms [7, 8, 11-14, 17, 21-23, 29, 30, 32].

Fungi that damage paper belongs to genera that have common ecological features since they are able to adapt to environmental conditions that are quite dry for micromycetes, since in most storages the relative humidity of the air does not exceed 50% and reaches a minimum during the heating season [2, 8] while colonizing paper documents. Micromycetes with cellulolytic, amylolytic, and glucosidase activity were repeatedly isolated from damaged library materials [10, 16, 19, 20, 26].

In turn, the abundance and availability of cellulose in the form of waste from agriculture, woodworking and pulp and paper industries make it an attractive raw material for the production of many biotechnologically valuable products, biodegradable polymeric materials, food products, including feed products enriched with protein and amino acids. For the complete hydrolysis of natural cellulose to bioavailable monomers, enzyme complexes are used, consisting mainly of endo-, exogluconase and cellobiase. Despite the fact that many researchers are engaged in the isolation of cellulolytic microorganisms, as well as genes [5, 15, 28, 31] responsible for the synthesis of these enzymes, the topic of searching for new, non-trivial ecological niches of possible producers of these enzymes remains relevant.

In the framework of this work, in order to assess the species richness and taxonomic structure of the biota, as well as to isolate strains that have the potential ability to degrade a cellulose-containing substrate, we examined documents from various institutions of St. State University (Faculty of Law and the Department of Rare Books), the libraries of the Alexander Nevsky Lavra, the Russian Geographical Society, the Russian State Library, the Department of Rare Books and Manuscripts of the Scientific Library of Moscow State University named after M.V. Lomonosov.

Samples were taken from documents on the surface of which spores, age spots, or damage of a supposed biological nature were found. Despite the species difference between the mycobiota of the air environment of book depositories and the surface of documents, many species are found in both ecological niches [8]. To compare the analysis of the ability of air micromycetes to synthesize cellulolytic enzymes, samples of the air of book depositories in the listed institutions were taken.

2 Method

2.1 Sample Selection

The number of microorganisms on the surface of documents was determined by the method of prints using wet sterile filter paper discs or cotton swabs [1, 3]. Sampling was carried out on the

Czapek-Dox nutrient medium and dichloroglycerol selective agar medium (DG 18). Cultivated for 5–14 days at (29±2)°C. Air samples were taken using a MAS-100 Eco aspirator.

2.2 Identification of Micromycetes

Micromycetes isolated from the air and from the surface of documents were isolated and identified on the basis of cultural and morphological features by light microscopy and the generally accepted technique of microscopy in transmitted light in a bright field under direct illumination on microscopes Olympus BX 53 M and Leica DM 2000, using the determinants of domestic and foreign authors [4, 9, 24, 25, 27]. The names of taxa are presented in accordance with the electronic database in the field of mycological nomenclature IndexFungorum. The presence of fungal species on the surface of documents was characterized by the frequency of occurrence [6].

2.3 Mycobiota Analysis

For the analysis of mycobiota, the following hierarchical ratios were used: species in the family (B/C), genera in the family (P/C), species in the genus (B/P), and species in the class (B/C). The mycobiots of the studied communities were analyzed using the Shannon species diversity index, Pilow and McIntosh evenness indices (DMc), Simpson and Berger-Parker dominance indices, and Mackintosh (U) and Menhinick species richness indices. Comparison of the species composition of micromycetes isolated from the surface of documents with the mycobiota of the air environment of storages was carried out using the qualitative similarity coefficients of Jaccard and Sørensen, and quantitative similarity coefficients of Sørensen and Morishita-Horn [18].

To identify micromycetes with cellulase activity, fungi were incubated on an agar medium with carboxymethylcellulose sodium salt (NaCMC) at a concentration of 10% as the sole carbon source. Incubation was carried out in a thermostat at a temperature of (29 ± 2) °C. On the third day, the medium was stained with Congo Red, the excess of which was washed off with 1 M NaCl solution. The interaction of this dye with a polysaccharide containing bonds β-1,4 or β-1,3, gives a complex of saturated red color. Microorganisms capable of destroying cellulose form clearing zones on plates with a substrate containing NaCMC.

The degree of cellulase activity was determined by the ratio of the surface area of the colony to the area of the lysis zone (lysis index), which characterizes the specific cellulase activity of the culture, since the area of the colony is proportional to its biomass, and the area of the lysis zone is proportional to the activity of the cellulase complex. Further, based on these data, the ranking of micromycete strains according to the activity of the cellulase complex was carried out.

2.4 Statistical Analysis

The obtained data on the species composition was processed by the method of multivariate statistics using the Statistica 13.3 software package. Means and standard deviations of colony area and lysis area were calculated using Microsoft Excel.

3 Result and Discussion

132 strains were isolated from the surface of documents in the libraries of St. Petersburg, and 43 strains from the air of storages, belonging to 52 and 25 species, respectively. The taxonomic structure of the mycobiota of the surface of library documents is represented by three divisions: Ascomycota, which occupies more than 90% of the species richness, Mucoromycota - 9%, and the smallest division Basidiomycota - 1%. In the air of the examined storage facilities, fungi from two divisions were present: Ascomycota - 98% and Mucoromycota - 2% (Table 1).

Table 1: The main divisions of micromycetes of the documents' surface and the air environment of storage.

Divisions	Share of micromycetes, %	
	On the documents' surface	In the air of storages
Ascomycota	90	98
Mucoromycota	9	2
Basidiomycota	1	-

Considering the taxonomic structure of the biota of the surface of documents, the following ratios of taxonomic hierarchies were revealed: V/S - 4.3, P/S - 1.3, V/P -3.3, V/K - 8.7. In the air storage facilities - 3.7; 1.4; 2.6; 5.2, respectively (Table 2).

In the spectrum of species richness of classes, both on the surface of documents and in the air of storages, the class Eurotiomycetes, the largest in terms of the number of species, is noted: it accounts for 66% and 77% of the total species richness, respectively, less saturated classes: Dothideomycetes - 10% and 11.5% and Sordariomycetes - 14% and 3.8%, respectively. Classes such as Mucoromycetes, Agaricomycetes, Saccharomycetes and Leotiomycetes are noted not in both ecological niches and in smaller numbers (Figures 1 and 2).

Table 2: The main divisions of micromycetes of the documents' surface and the air environment of storage.

Family name	Species richness	
	On the documents' surface	In the air of storage
Aspergillaceae	33.0	76.9
Cladosporiaceae	2.0	3.8
Dipodascaceae	2.0	-
Hypocreaceae	6.0	3.8
Mucoraceae	-	3.8
Nectriaceae	-	2.0
Phanerochaetaceae	-	4.0
Plectosphatrellaceae	-	2.0
Pleosporaceae	6.0	3.8
Rhizopodaceae	4.0	-
Sclerotiniaceae	-	3.8
Stachybotryaceae	2.0	-
Torulaceae	2.0	3.8

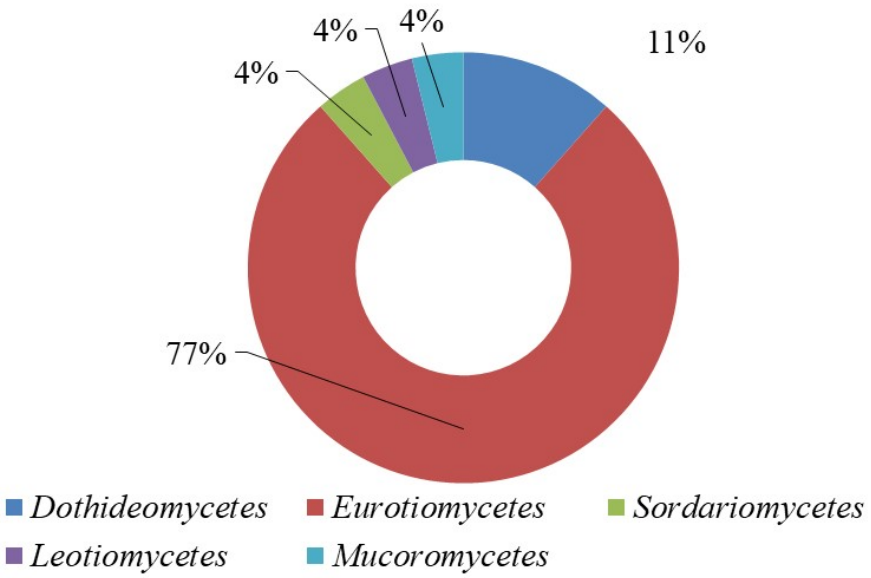

Figure 1: Species richness of storage air micromycete classes

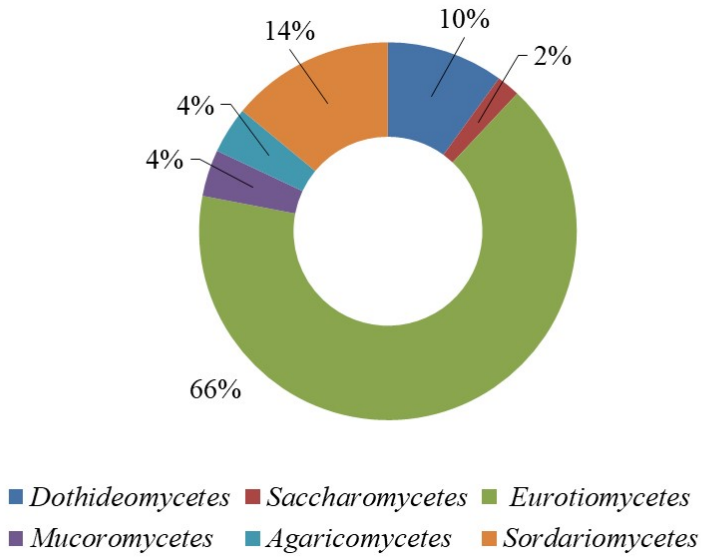

Figure 2: Species richness of classes of micromycete surface papers

Further analysis of the taxonomic groups revealed 12 families on the surface of the documents and seven in the air of the vaults. The dominant species in the spectrum of species richness of both ecological niches is the Aspergillaceae family, which accounts for 33% and 77%, respectively, of the total species richness. In the second place, but by a significant margin, are the Pleosporaceae and Hypocreaceae families. Species of the families Dipodascaceae, Rhizopodaceae, and Stachybotryaceae were found only on the surface of documents, while the families Mucoraceae, Nectriaceae, Phanerochaetaceae, Plectosphatrellaceae, and Sclerotiniaceae were found only in the air of storage facilities.

On the surface of the documents of the libraries of St. Petersburg and Moscow, fungi of 16 genera were identified, from the air - 10. The leader was the genus Penicillium, which accounted for 46% of the total number of species on the surface of documents and 35% in the air of storages, followed by Aspergillus - 14% on the surface documents and 23.1% in air storages, Talaromyces

accounted for 5.8% and 13.5%, respectively. On the surface of documents and in the air of storages, the genera Alternaria, Cladosporium, Paecilomyces, Rhizopus, Torula, and Trichoderma occupied from 2 to 6% of the total species richness. Species of the genera Chaetomium, Dipodascus, Fusarium, Mucor, Sporotrichum, Stachybotrys, Verticillium were found only on the surface of documents, and the genus Botrytis only in the air of storages.

An assessment of the indices of species diversity of micromycetes on the surface of documents and in the air of storage showed that it is moderately high: the Shannon index is 3.27 and 2.71, respectively. The Simpson dominance index is low - 0.054 and 0.087, which is confirmed by the alternative Berger-Parker dominance index - 0.13 and 0.21, which shows the degree of dominance of the most widespread species. A high level of evenness of species in the mycobiota is evidenced by a rather high Pielow index - 0.83 and 0.84 for the surface of documents and storage air, respectively. The Macintosh Dominance Index, which is not affected by sample size, also confirms the high level of species evenness of 0.81 and 0.78 for document surface and storage air. The Macintosh Diversity Index indicates a significant richness of species on the surface of library documents - 71.9, and much lower in the air of vaults - 22.8. Having calculated the Manhinick index, which is not affected by the sample size, we obtained almost the same values for the surface of library documents and storage air: 2.88 and 2.95, respectively (Table 3).

Table 3: Ecological diversity of micromycetes on the surface of library documents.

Diversity indicators	On the documents' surface	In the air of storages
Total species	52	25
Simpson index	0.054	0.087
Pielow's evenness index	0.83	0.84
Shannon index	3.27	2.71
Macintosh index	71.9	22.8
Macintosh dominance index	0.81	0.78
Berger-Parker index	0.13	0.21
Menhinick index	2.88	2.95

Binary coefficients of similarity for the studied ecological niches revealed a rather high species difference between them (Table 4).

Table 4: Ecological diversity of micromycetes on the surface of library documents and storage air.

Measure of diversity	Value
Jaccard measure	0.28
Sorensen measure (qualitative data)	0.44
Sorensen measure (quantitative data)	0.28
Morishita-Horn measure)	0.57

Using the cluster analysis method, based on the frequency of occurrence and abundance of species for each studied ecological niche, complexes of micromycetes were formed, consisting only of their characteristic species. Both complexes consist only of frequently occurring species since there are no dominant species, which is confirmed by the low values of the Simpson and Berger-

Parker dominance indices. The complex of micromycetes, characteristic of the surface of documents in the libraries of St. Petersburg and Moscow, consists of three clusters: the first includes the species Aspergillus niger and Penicillium aurantiogriseum with a frequency of occurrence of 14.7% and 13.9%, respectively; in the second Penicillium canescens (8.9%), in the third - Rhizopus stolonifer (6.6%), Penicillium lanosocoeruleum (6.2%), Penicillium lanosum (6.2%) and Chaetomium globosum (5.8%) (Figure 3).

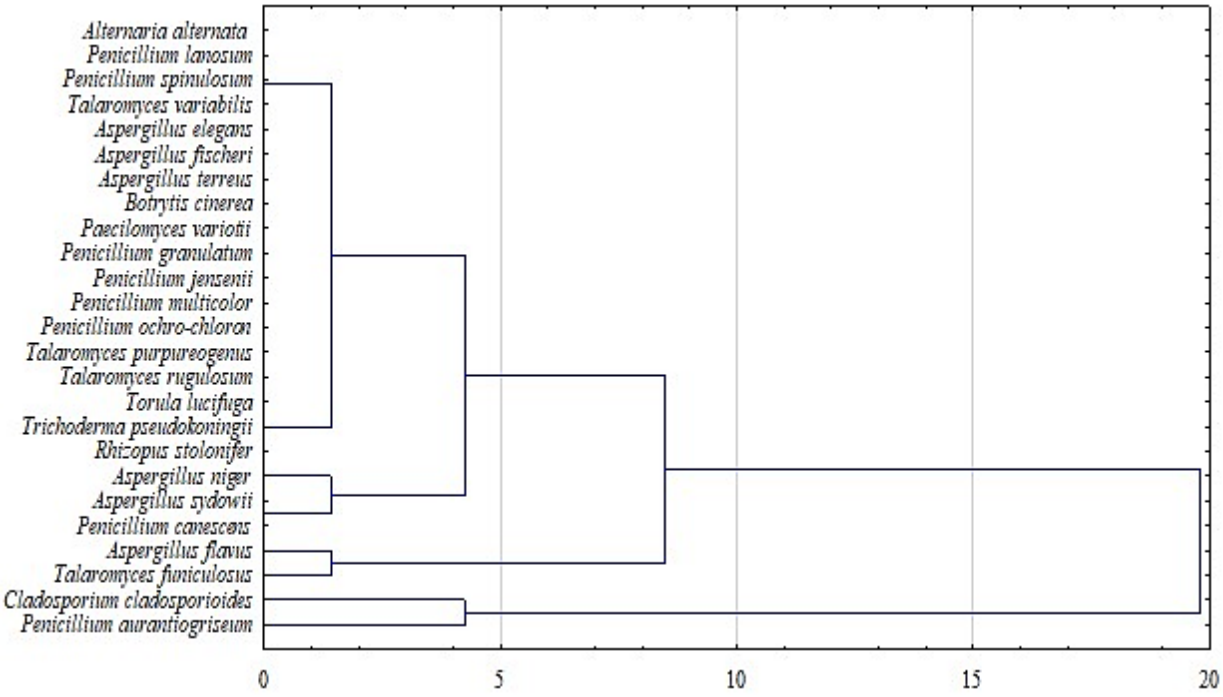

Figure 3: Cluster analysis based on the occurrence' frequency and abundance of species isolated from the documents' surface.

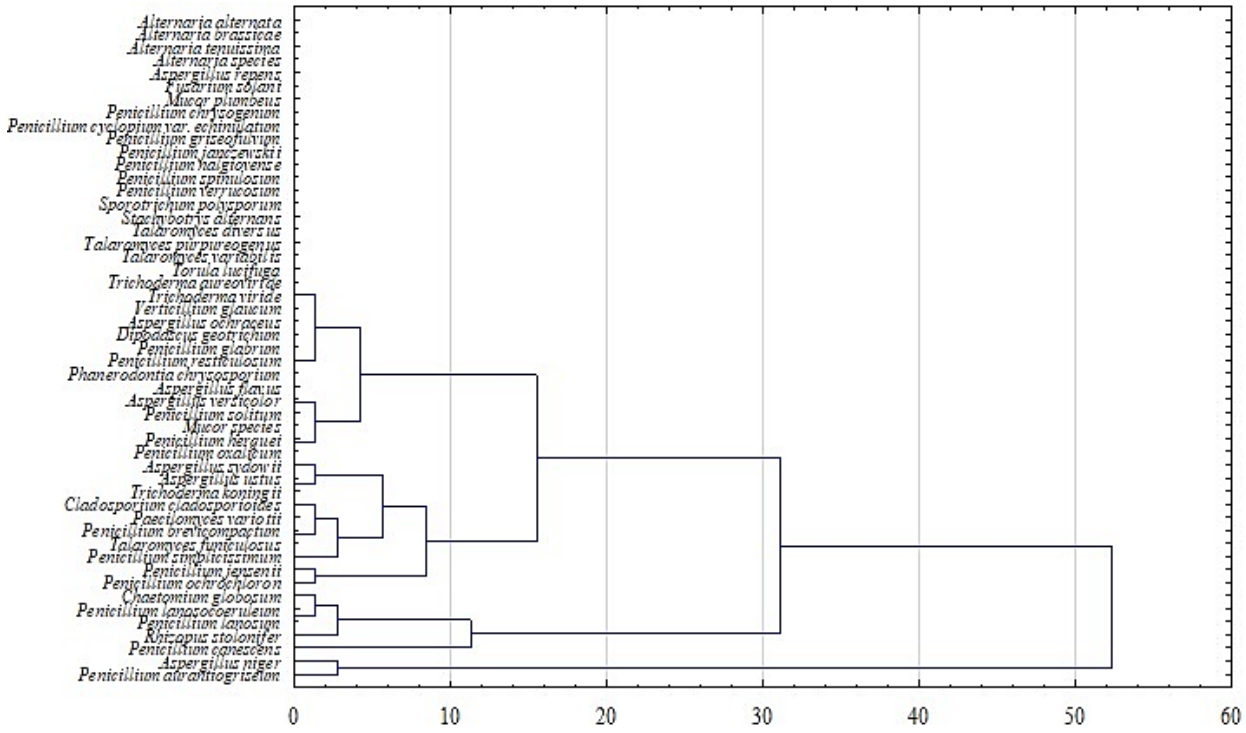

Figure 4: Cluster analysis based on the frequency and abundance of species isolated from storage air

The complex of micromycetes characteristic of the air in St. Petersburg libraries consists of two clusters: the first is represented by the species Cladosporium cladosporioides (40.5%) and Penicillium aurantiogriseum (32.4%), the second - by Aspergillus flavus (18.9%) and Talaromyces funiculosus (16.2%) (Figure 4). It is noteworthy that only P. aurantiogriseum was included in both assemblages.

During the growth process fungi can release cellulolytic enzymes, therefore, in all isolated strains isolated both from the surface of documents (paper) and from the air of book storages, the presence of cellulose-degrading activity was determined.

For a qualitative assessment of the degree of cellulolytic activity of micromycetes, the following designations were adopted: "-" - activity was not registered; "+" - weak; "++" - medium; "+++" - strong cellulolytic activity (Table 5).

Table 5: Species composition of micromycetes isolated from the surface of documents.

	Types of micromycetes	Strain number	Frequency of occurrence	Cellulolytic activity
1	Alternaria alternata (Fr.) Keissl.	X1	0.4	-
2	Alternaria brassicae (Berk.) Sacc.	K1	0.4	-
3	Alternaria tenuissima (Kunze) Wiltshire	X2	0.4	-
4	Alternaria species	K2	0.4	-
5	Aspergillus flavus Link	K3	1.9	-
6		K4		-
7		X3		-
8		X4		+
9		D2		-
10	Aspergillus niger Tiegh.	K5	14.7	-
11		K6		-
12		K7		-
13		K8		+
14		K9		-
15		K10		-
16		K11		-
17		K12		-
18		K13		-
19		X5		-
20		X6		-
21	Aspergillus ochraceus G. Wilh.	X7	0.8	-
22		D4		-
23	Aspergillus repens (Corda) Sacc.	K14	0.8	+
24		D1		+
25	Aspergillus sydowii (Bainier et Sartory) Thom et Church	K15	2.3	+++
26		X8		+++
27		X9		+++
28		X10		++
29	Aspergillus ustus (Bainier) Thom et Church	K16	2.7	++
30		K17		++
31		X11		-
32		X12		-
33	Aspergillus versicolor (Vuill.) Tirab.	K18	1.5	+
34		X13		++
35		X14		+
36		D16		+
37	Chaetomium globosum Kunze	K19	5.8	+
38		K20		+
39		X15		+
40		X16		+
41		X17		+
42		X18		+++

	Types of micromycetes	Strain number	Frequency of occurrence	Cellulolytic activity
43		D5		+
44	Cladosporium cladosporioides (Fresen.) G.A. de Vries	K21	3.5	+
45		K22		++
46		X19		-
47		X20		-
48		X21		+
49		X22		+++
50	Fusarium solani (Mart.) Sacc.	D23	0.4	+
51	Dipodascus geotrichum (E.E. Butler et L.J. Petersen) Arx	K53	0.8	++
52		K54		++
53	Mucor plumbeus Bonord.	D22	0.4	+
54	Mucor species	X51	1.2	+
55	Paecilomyces variotii Bainier	X23	3.1	-
56		K23	2.95	-
57	Penicillium aurantiogriseum Dierckx	K24	13.9	+
58		K25		+
59		K26		+
60		X24		+
61		X25		+
62		X26		+++
63		X27		+
64		X28		++
65		X29		+
66		X30		+++
67	Penicillium brevicompactum Dierckx	K27	3.1	+
68		K28		++
69		D6		+
70	Penicillium canescens Sopp	K29	8.9	+++
71		X31		+++
72		X32		++
73	Penicillium chrysogenum Thom	D7	0.4	++
74	Penicillium cyclopium var. echinulatum Raper et Thom	X33	0.4	+
75	Penicillium glabrum (Wehmer) Westling	K30	0.8	++
76		D8		++
77	Penicillium griseofulvum Dierckx	X34	0.4	+
78	Penicillium herquei Bainier et Sartory	X35	1.2	+
79		D9		+
80	Penicillium janczewskii K.W. Zaleski	D10	0.8	+
81	Penicillium jenseni K.W. Zalessky	K31	4.2	+
82		K32		+
83		X36		++
84		D11		+
85	Penicillium lanosocoeruleum Thom	X37	6.2	+
86		X38		-
87	Penicillium lanosum Westling	K33	6.2	+
88		K34		+
89		X39		+
90		X40		+
91	Penicillium nalgiovense Laxa	D12	0.4	-
92	Penicillium ochrochloron Biourge	K35	4.6	+
93		D13		++
94	Penicillium oxalicum Currie et Thom	X41	1.2	+
95	Penicillium resticulosum Birkinshaw, Raistrick et G. Sm.	K36	0.8	+
96	Penicillium simplicissimum (Oudem.) Thom	K37	3.9	+
97		K38		+++
98		X42		++
99		X43		-
100	Penicillium solitum Westling	K39	1.5	++
101		D14		++
102	Penicillium spinulosum Thom	D15	0.4	+

	Types of micromycetes	Strain number	Frequency of occurrence	Cellulolytic activity
103	Penicillium verrucosum Dierckx	K40	0.4	-
104	Phanerodontia chrysosporium (Burds.) Hjortstam et Ryvarden	K56	0.8	+
105		X52		-
106	Rhizopus stolonifer (Ehrenb.) Vuill.	K55	6.6	+
107		X48		+
108		X49		+
109		X50		+
110	Sporotrichum polysporum Link	D21	0.4	-
111	Stachybotrys alternans Bonord.	X44	0.4	+
112	Talaromyces diversus (Raper et Fennell) Samson, N. Yilmaz et Frisvad	K41	0.4	+
113	Talaromyces funiculosus (Thom) Samson, N. Yilmaz, Frisvad et Seifert	K42	3.1	-
114		K43		+
115		K44		+++
116		K45		++
117		K57		+
118		K58		-
119		D17		-
120	Talaromyces purpureogenus (Stoll) Samson, N. Yilmaz, Houbraken, Spierenb., Seifert, Peterson, Varga et Frisvad	D18	0.4	+
121	Talaromyces variabilis (Sopp) Samson, N. Yilmaz, Frisvad et Seifert	K46	0.8	-
122		D20		-
123	Torula lucifuga Oudem.	K47	0.4	+
124	Trichoderma aureoviride Rifai	K48	0.4	-
125	Trichoderma koningii Oudem.	K49	2.7	-
126		K50		-
127		X45		-
128		X46		-
129		X47		-
130		D19		-
131	Trichoderma viride Pers.	K51	0.4	-
132	Verticillium glaucum Bonord.	K52	0.4	-

- — activity is not registered
+ - weak cellulolytic activity
++ - average cellulolytic activity
+++ - strong cellulolytic activity

The strains isolated from the surface of the documents (65%) had the ability to degrade the cellulose of the nutrient medium (they gave a clearing zone when stained with Congo red). The cellulolytic activity of most of these strains (64%) is conditionally characterized as weak, 22% as a medium, and only 14% as strong. Only in half of the strains isolated from the storage air, the activity of the cellulolytic complex was registered, which in more than half of the cases was defined as weak (Table 6).

Table 6: Species composition of micromycetes isolated from storage air.

	Types of micromycetes	Strain number	Frequency of occurrence	Cellulolytic activity
1	Alternaria alternata (Fr.) Keissl.	B1	5,4	-
2	Aspergillus elegans Gasperini	B2	2,7	-
3	Aspergillus flavus Link	B3	18,9	-
4		B4		-
5		B5		-
6	Aspergillus fischeri Wehmer	B6	2,7	-
7	Aspergillus niger Tiegh.	B7	10,8	-
8		B8		++
9		B9		-

10	Aspergillus sydowii (Bainier et. Sartory) Thom et. Church	B10	8,1	-
11		B11		-
12	Aspergillus terreus Thom	B12	2,7	+
13	Botrytis cinerea Pers.	B13	2,7	No data
14	Cladosporiumcladosporioides (Fresen.) G.A. de Vries	B14	40,5	+
15		B15		+
16		B16		-
17		B17		+
18	PaecilomycesvariotiiBainier	B18	2,7	-
19	Penicillium aurantiogriseum Dierckx	B19	32,4	+
20		B20		++
21		B21		+
22		B22		+
23		B23		-
24	Penicillium canescens Sopp	B24	8,1	++
25	Penicillium granulatum Bainier	B25	2,7	-
26	Penicillium jensenii K.W. Zalessky	B26	2,7	+
27	Penicillium lanosum Westling	B27	5,4	+
28		B28		++
29	Penicillium multicolor Grig.-Man. et Porad.	B29	2,7	-
30	Penicillium ochrochloron Biourge	B31	2,7	++
31	Penicillium spinulosum Thom	B32	5,4	+
32		B33		+
33	Rhizopus stolonifer (Ehrenb.) Vuill.	B44	2,7	++
34	Talaromyces funiculosus (Thom) Samson, N. Yilmaz, Frisvad et Seifert	B34	16,2	+
35		B35		+
36		B36		-
37		B37		++
38		B38		-
39	Talaromyces purpureogenus (Stoll) Samson, N. Yilmaz, Houbraken, Spierenb., Seifert, Peterson, Varga et Frisvad	B39	2,7	++
40	Talaromyces rugulosus (Thom) Samson, N. Yilmaz, Frisvad et Seifert	B40	2,7	+
41	Talaromyces variabilis (Sopp) Samson, N. Yilmaz, Frisvad et Seifert	B41	5,4	-
42	Torula lucifuga Oudem.	B42	2,7	No data
43	Trichoderma pseudokoningii Rifai	B43	2,7	No data

When compared with micromycetes isolated from the surface of documents, cellulase activity in strains from storage air is significantly lower.

Different strains of the same species isolated from similar substrates may have different activities of the enzyme complex. Thus, among strains of Aspergillus ustus, Cladosporium cladosporioides, Talaromyces funiculosus, Penicillium simplicissimum, Phanerodontia chrysosporium species, both representatives with medium and high cellulase activity, as well as strains in which this enzymatic activity was not recorded, were identified. Two species can be noted: Aspergillus sydowii and Penicillium canescens, in which all isolated strains had consistently high cellulase activity.

In total, 11 strains of the following species were classified as active cellulolytic: Penicillium canescens, Penicillium aurantiogriseum, Penicillium simplicissimum, Aspergillus sydowi, Talaromyces funiculosus, Chaetomium globosum, Cladosporium cladosporioides. Three species are representatives of the complex of micromycetes characteristic of the surface of documents, and two species are representatives of the air environment of library book depositories.

4 Conclusion

The study gave an idea of the taxonomic structure of the mycobiota of documents and air in the libraries of St. Petersburg and Moscow showing that 65% of the strains of these two biotas have cellulase activity. It has also been shown that micromycetes isolated from the surface of documents are more promising cellulolytic: strains with the highest activity have been isolated from this ecological niche. These strains were selected for the subsequent quantitative determination of the activity of the components of the cellulase complex and the detection of genes responsible for their synthesis.

5 Availability of Data and Material

Data can be made available by contacting the corresponding author.

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