МИКОЛОГИЯ И ФИТОПАТОЛОГИЯ, 2023, том 57, № 5, с. 321–331

БИОРАЗНООБРАЗИЕ, СИСТЕМАТИКА, ЭКОЛОГИЯ

УДК 573.7 + 550.72

THE PECULIARITIES OF THE MYCOBIOTA FORMATION ON THE SAINT PETERSBURG STONE MONUMENTS BASED ON METAGENOMICS AND CULTURAL DATA

© 2023. K. V. Sazanova[1,2,3,*], M. S. Zelenskaya[2,], O. A. Pavlova[4,***], and D. Yu. Vlasov[1,2,****]**

[1]*Komarov Botanical Institute of the Russian Academy of Sciences, 197022 St. Petersburg, Russia*

[2]*St. Petersburg State University, 199034 St. Petersburg, Russia*

[3]*Archive of the Russian Academy of Sciences, St. Petersburg Branch, 196084 St. Petersburg, Russia*

[4]*Beagle LTD Company, St. Petersburg, 192289 Russia*

**e-mail: ksazanova@binran.ru*

***e-mail: marsz@yandex.ru*

****e-mail: beagle07@bk.ru*

*****e-mail: dmitry.vlasov@mail.ru*

Received April 15, 2023; revised May 20, 2023; accepted May 23, 2023

Fungi play an important role in colonization and biodeterioration of stone monuments in the open air. This study significantly complements the data on fungal diversity in lithobiotic communities through the use of metagenomic analysis. It was shown that the mycobiota of tombstones in the historical center of St. Petersburg has a complex structure. There are different complexes of species, which have various origins and ways of getting to the monuments. The complex of dominant fungi in SABs on marble and granite in St. Petersburg is formed by dark-colored micromycetes. At the species level, the absolute dominant in all samples was the *Knufia karalitana* according to metagenomic data and *Aureobasidium pullulans* according to cultural data. The use of two methodological approaches indicates the expediency of combining culture-based and molecular genetics methods, which make it possible to obtain the most more complete picture of the formation of lithobiotic communities. The season and the type of biofilm have a key importance for the abundance and diversity of micromycetes on stone surface. It was shown that type of rock has a minimal importance for the fungal diversity on stone monument.

Keywords: cultivation and metagenomic methodologies, cultural heritage, fungal diversity, microbial community, microfungi, subaerial biofilms

DOI: 10.31857/S0026364823050070, **EDN:** YECIMP

INTRODUCTION

Cultural heritage preservation is an important problem for human society (Villa et al., 2016; Liu et al., 2020). Stone cultural heritage has a great historical, artistic and scientific value. Most of stone monuments are destroyed due to physical, chemical and biological influences over time.

Natural stone is important ecosystems providing an ecological niche for various microorganisms (Zhang et al., 2023). Fungi, algae, bacteria, mosses and lichens are common for stone surface in different geographic locations. They form the lithobiotic communities on the monuments, embankments, facades of the historical building and often cover a significant surface of stone. Stone monuments may be degraded by growth and activity of living organisms. Their interaction with stone surface is one of the main factors of the cultural heritage biodeterioration processes (Villa et al., 2016; Zhang et al., 2023). Microbial communities at the stone-air border are called subaerial biofilms (SABs) (Gorbushina, 2007; Liu et al., 2020; De Leo et al., 2022; Zhang et al., 2023).

Microfungi are typical inhabitants of the SABs on any type of stone. A number of researchers consider the activity of microfungi leads to damage of natural and artificial stone (Kurakov et al., 1999; Gorbushina et al., 2002; Onofri et al., 2014; De Leo, Urzi, 2015; Salvadori, Municchia, 2016; Isola et al., 2016; Kirtsideli et al., 2016; Isola et al., 2022; De Leo et al., 2022).

Deterioration caused by fungi involves both physical and chemical effects (simultaneously) on stone substrates (De Leo, Urzi, 2015; Sazanova et al., 2020). Cracks, cavities, pores and fissures facilitate penetration of microorganisms and provide favorable microenvironments on stone surfaces. Melanin and extracellular polymeric substances (EPS) produced by fungi promote biofilm formation and adherence to stone surface and increase mechanical pressure causing swelling (Burford et al., 2003; Gorbushina, 2007).

Table 1. Characteristics of samples

Sample	Type of biofilm	Stone	Monument and location in The Museum necropolis
1	SAB 1: dark-coloured biofilms with domination of non-lichenized fungi	White Carrara homogeneous marble	Monument to Unknown in The Museum Necropolis of 18th Century
2		Rapakivi granite (pink ovoid granite)	Monument to V.V. Stasov in the Museum Necropolis of Masters of Art
3	SAB 2: green biofilms with domination of algae	White Carrara homogeneous Marble	Monument to A.A. Borozdin in The Museum Necropolis of 18th Century
4		Serdobol granite (grey homogeneous granite)	Monument to I.A. Olchin in The Museum Necropolis of 18th Century
5	SAB 3: biofilms with domination of lichens	White Carrara homogeneous marble	Monument to A.N. Bukarevskaya in The Museum Necropolis of 18th Century
6		Serdobol granite (grey homogeneous granite)	Monument to P.E. Nikitin in The Museum Necropolis of 18th Century

Undoubtedly, the data about the fungal biodiversity directly depends on the methodology of the study. For many years, methods for studying microorganisms inhabiting cultural heritage have been culture-dependent, associated with the ability of microbes to grow under solid-phase cultivation at the laboratory, and the consequent use of microscopy technique. Such approach makes it possible to visualize and preserve cultures for further study of their physiology and metabolism (Paiva et al., 2022). However they provide limited information on the diversity of fungal communities. This approach has certain limitations and only reveals a small part of the total diversity because some microfungi are not growing on nutrient media (González, Sáiz-Jiménez, 2005; Dakal, Arora, 2012; Mihajlovski et al., 2015). In addition, very often cultivation conditions are favorable for some species and hinder the development of others.

The culture-independent methods based on DNA analysis provide most complete information on the composition of microbial communities. Such methods are increasingly used in recent years (González, Sáiz-Jiménez, 2005; Trovão et al., 2019; Paiva et al., 2022; Zhang et al., 2023). In recent studies, the authors have emphasized the importance of integrating metagenomics and cultivations approaches. Such methodology provides a clear understanding of the actual fungal biodiversity on stone surface, as well as to keep the strains to study their potential destructive importance (Onofri et al., 2014; Zhang et al., 2023).

Climate and microenvironment are important factors influencing the colonization of organisms (De Leo, Urzi, 2015). The unique collection of monuments made of marble, limestone and granite is located in Saint Petersburg and undergoes intensive biological colonization. Studies on the microfungi diversity on historical monuments have been carried out in Saint Petersburg since 1998. More than 300 outdoor monuments were examined during this period. The focus was made on the tombstones of the Museum necropolises of the State Museum of Urban Sculpture of Saint Petersburg. The biodiversity of microfungi on Saint Petersburg stone monuments was studied previously by cultivation methods (Vlasov et al., 2002; Gorbushina et al., 2002; Sazanova et al., 2020, 2022). However, some aspects of the fungal biodiversity study at sites still remain unexplored. The first question is how the picture of fungal biodiversity in biofilms on sculptural monuments will change when using metagenomic analysis in comparison with cultural data. The second question is how the composition of fungi on monuments changes during one growing season, which is important when developing methods for protecting monuments against bioweathering. In this work, we have tried to answer these questions.

MATERIALS AND METHODS

Sampling. Samples for this study were taken in two seasons of 2022: early vegetation season (April) and late vegetation season (September). Material for the study was collected at the territory of Museum necropolis of 18th Century and the Necropolis of Masters of Art (State Museum of Urban Sculpture, central part of Saint Petersburg). The tombstones made of silicate (granite) and carbonate (marble) rocks were included in the research. Samples for cultivation and metagenomic analysis were taken from various types of SABs: dark-coloured biofilms, presumably formed mainly by non-lichenized fungi (SAB 1); green biofilms with algae domination (SAB 2), and biofilms with lichens domination (SAB 3) (Table 1, Fig. 1). Samples of biofilms for cultivation and metagenomic analysis were collected from the same locations on tombstones and places into sterile containers.

Fungal isolation and identification. For the primary isolation, cultivation, and identification of microfungi small fragments of biofilms were placed on the surface

Fig. 1. Types of studied SABs and details of the sampling areas on tombstones: a – territory of Museum Necropolis; b – dark-colored biofilms with domination of fungi (SAB1) on white Carrara marble (monuments to Unknown); c – green biofilms with domination of algae (SAB2) on white Carrara marble (Monument to A.A. Borozdin); d – biofilms with domination of lichens (SAB3) on white Carrara marble (Monument to A.N. Bukarevskaya).

Czapek – Dox nutrient medium and incubated for 2–4 weeks at a temperature of 22°C. Identification was carried out in accordance with morphological characteristics. The frequency of species occurrence (number of species findings in all samples, %) was also detected. The abundance of species was account based on the ratio of the number of species colonies to the total number of colonies on a nutrient medium.

Metagenomic analysis. DNA isolation was performed according to the method described by Vladimirov et al. (2014), additional purification in low-melting agarose was used. Commercial PowerSoil DNA Isolation kit (MO BIO Laboratories, Inc, Carlsbad, CA, USA) were used to isolate DNA. We had to use additional purification to remove humic acid pollution. Identification of microfungi in biofilms was carried out using primers for amplification of the site (ITS1–5.8 S–ITS2) (Beagle, St. Petersburg, Russia). Primers for the analysis of maximum diversity were used from the guide (Fungal sequencing., 2018). Amplification was carried out using the FastStartHighFidelityPCRsystem kit (Roche, Basel, Switzerland). Amplicons for library preparation were obtained by thermal cycling according to the protocol: 3 min at 95°C, 30 cycles (30 s at 95°C, 30 s at 55°C, 40 s at 72°C). Sample preparation was performed according to the standard protocol (Fungal metagenomic sequencing., 2019). Sequencing was performed on a MiSeq instrument using a MiSeqReagentKit v3 (600-cycle) in pair-ended mode 2 × 300 cycles. The data obtained were analyzed using the QIIME2 software package. When forming the OTU, a sequence similarity threshold of 97% was used.

RESULTS

Cultivated fungal diversity

As a result of cultural studies 19 species of *Ascomycota* were identified (Table 2). Only one species of *Mucoromycota* (*Entomortierella lignicola*) was found in one sample. *Aureobasidium pullulans*, *Alternaria alternata* and *Cladosporium cladosporioides* were found in more than 90% of samples. The occurrence of *Alternaria chartarum*, *Cladosporium herbarum*, *C. sphaerospermum* and *Sydowia polyspora* reached to 50%. The abundance of *Aureobasidium pullulans* amounted to 85%.

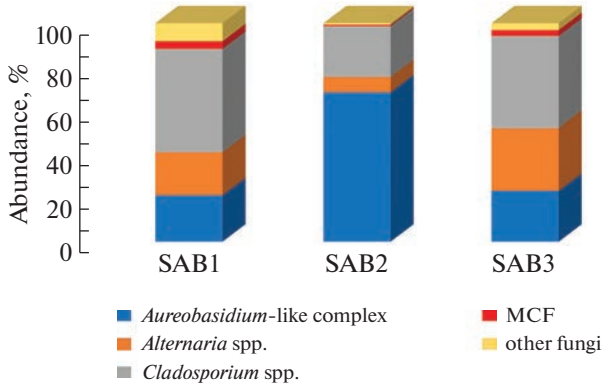

Fig. 2. Abundance of fungi in samples of different types of SABs (the data combined for different seasons) according to culture-dependent studies.

The average abundance of *Cladosporium cladosporioides* was about 20%, and in some samples it reached 50%. The abundance of *C. herbarum* and *C. sphaerospermum* varied from 10 to 23%. The average abundance of *Alter-naria alternata* was 15–25%. The abundance of *A. chartarum* reached 13% but was generally less than 10%. The abundance of *Sydowia polyspora* averaged 5%.

Some groups of species clearly dominate on the surface of the stone during vegetation period of 2022. The first group is presented by fungi with *Aureobasidium*-like morphology: *Aureobasidium pullulans*, *Sydowia polyspora* and *Exophiala exophialae*. Super-dominant in all samples is *Aureobasidium pullulans*. The second group includes species of genus *Alternaria* (*A. alternata* and *A. chartarum*). The third group is formed by *Cladosporium* spp. complex (*C. cladosporioides*, *C. herbarum* and *C. sphaerospermum*). The abundance of MCFs (microcolonial fungi) reached 14%. Other fungi were less abundant in SABs.

About a quarter of the total diversity consists of typical anamorphic soil fungi: *Trichoderma viride*, *Botrytis cinerea*, *Fusarium oxysporum*, *F. solani*, *Tritiraci-um* sp. and *Epicoccum nigrum*. In general their abundance was 1.5–2%. The genus *Penicillium* was represented by four species: *Penicillium brevicompactum*, *P. chrysogenum*, *P. decumbens, P. oxalicum*. These species were founded rarely and its abundance did not exceed 2.5%.

Comparative analysis of the biodiversity of fungi in different types of SABs (Fig. 2) showed that the samples of SAB2 contrasted with SAB1 and SAB3 due to the maximum abundance of *Aureobasidium*-like fungi, primarily *Aureobasidium pullulans* in SAB2 (up to 88% SAB2 and up to 50% in SAB1 and SAB3). MCFs were found predominantly in SAB1 and SAB2.

Significant differences of fungal diversity were associated with the season (Fig. 3). The general patterns of changes in the structure of fungal communities were an increase in biodiversity and relative abundance of rare species with a decrease in the abundance of the dominant species *A. pullulans* in the autumn period. The species diversity and abundance of microfungi in SAB2 were significantly lower than in SAB1 and SAB3 in the spring period. But in autumn samples these parameters were about the same. There were no significant differences in the diversity of fungi between silicate and carbonate rocks (Fig. 3).

Metagenomic analysis of fungal diversity. As a result of metagenomics data the diversity of fungi mainly represented by *Ascomycota* – 71–97%. The share of *Basidiomycota* is 0.2–14.3%. About 10% of total DNA in the all samples was not identified at the range of division. As the rank of the taxon decreased, the number of unidentified fungi increased and reached about 20% at the genus level. For a detailed description and comparative analysis we have chosen orders and genera. This choice is due to the greatest visibility of the distribution of fungi according to taxonomic affiliation (at the level of orders) and the overall diversity of fungi (at the level of genera) between the compared samples.

The number of orders varied for different samples and reached 61 (Fig. 4, a, b). Among them six orders

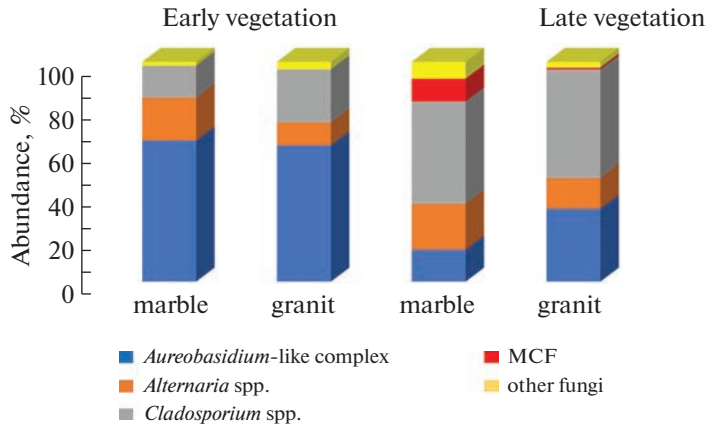

Fig. 3. The abundance of microfungi in samples from the surface of various rocks at different seasons.

Table 2. Microfungi from subaerial biofilms (SABs) on marble and granite (culture-based method)

Species	Frequency of occurrence, %	Early vegetation season						Late vegetation season					
		Marble			Granite			marble			granite		
		SAB1	SAB2	SAB3	SAB 1	SAB2	SAB3	SAB1	SAB2	SAB3	SAB1	SAB2	SAB3
Alternaria alternata (Fr.) Keissl.	92	+	+	+	+	–	+	+	+	+	+	+	+
A. chartarum Preuss	50	–	+	+	–	–	–	+	+	+	–	–	+
Aureobasidium pullulans (de Bary et Löwenthal) G. Arnaud	92	+	+	+	+	+	+	+	+		+	+	+
Botrytis cinerea Pers.	17	–	–	–	–	–	–	–	–	–	+	+	–
Cladosporium cladosporioides (Fresen.) G.A. de Vries	92	+	+	–	+	+	+	+	+	+	+	+	+
C. herbarum (Pers.) Link	50	+	–	–	+	–	–	+	+	+	–	+	–
C. sphaerospermum Penz.	42	–	–	–	–	–	+	+	–	+	+	–	+
Entomortierella lignicola (G.W. Martin) Vandepol et Bonito	8	–	–	–	–	–	–	–	–	–	–	–	+
Epicoccum nigrum Link	8	+	–	–	–	–	–	–	–	–	–	–	–
Exophiala exophialae (de Hoog) de Hoog	8	–	–	–	–	–	–	–	–	+	–	–	–
Fusarium oxysporum Schltdl.	17	–	–	–	+	–	–	+	–	–	–	–	–
F. solani (Mart.) Sacc.	8	+	–	–	–	–	–	–	–	–	–	–	–
Penicillium brevicompactum Dierckx	17	–	–	–	+	–	–	–	–	–	+	–	–
P. chrysogenum Thom	8	+	–	–	–	–	–	–	–	–	–	–	–
P. decumbens Thom	17	–	–	+	–	–	–	–	–	+	–	–	–
P. oxalicum Currie et Thom	8	–	–	–	–	–	–	–	–	–	+	–	–
Sydowia polyspora (Bref.) E. Müll.	50	–	+	+	+	–	+	–	–	–	+	–	+
Trichoderma viride Pers.	8	–	–	–	–	–	+	–	–	–	–	–	–
Tritiracium sp.	8	–	–	–	–	–	–	–	–	–	–	+	–
MCF (microcolonial fungi)	25	–	–	–	–	–	–	+	–	+	–	+	–

Table 3. Main genera of fungi in SABs according to metagenomic analysis

Genus/Share in metagenome, %	Early vegetation season						Late vegetation season					
	marble			granite			marble			granite		
	SAB 1	SAB 2	SAB 3	SAB 1	SAB 2	SAB3	SAB 1	SAB2	SAB3	SAB1	SAB2	SAB3
Ascomycota												
Knufia	70.4	75.8	71.4	29.3	83.8	84.9	62.9	54.1	63.5	1.3	84.4	41.3
Aureobasidium	10.3	16.9	22.2	51.8	0.7	0.6	1.1	–	–	1.6	4.3	–
Capronia	–	1.2	3.1	5.4	13.0	2.6	1.2	1.8	6.6	–	3.9	15.8
Cladosporium	–	–	–	–	–	–	–	1.0	–	19.6	–	–
Rhinocladiella	–	–	–	7.1	–	9.9	–	–	6.6	–	–	11.9
Celosporium	–	0.9	0.3	2.8	–	0.6	1.8	–	–	1.0	–	4.6
Neomicrosphaeropsis	–	–	–	–	–		3.5	3.4	–	–	–	–
Vermiconia	0.5	–	–	–	–	–	1.6	–	6.4	–	–	–
Acericola	–	0.7	–	–	–	0.7	1.2	–	1.8	–	–	1.2
Candida	–	–	–	–	–	–	–	0.5	2.3	2.9	–	–
Exophiala	–	–	0.8	–	–	–	–	4.1	–	–	0.5	–
Penicillium	0.8	–	–	0.7	–	–	0.2	1.8	0.7	4.6	–	–
Alternaria	2.5	0.1	–	–	0.1	–	1.1	0.3	–	3.8	–	–
Epicoccum	–	–	–	–	–	–	1.0	–	–	1.7	–	–
Aspergillus	1.1	–	–	–	–	–	–	–	–	1.3	–	–
Trichomerium	–	–	–	–	–	–	–	1.4	3.6	0.9	–	5.1
Coniosporium	–	–	–	–	–	0.3	0.9	–	1.6	–	–	2.0
Paracamarosporium	–	–	0.1	0.4	0.1	0.3	0.3	1.0	0.9	–	0.9	4.4
Stemphylium	–	–	–	–	–	–	–	1.2	–	0.6	–	–
Tolypocladium	–	–	–	–	–	–	–	0.8	–	–	–	2.9
Basidiomycota												
Vishniacozyma	–	–	–	–	–	–	2.5	–	–	4.4	–	–
Naganishia	–	–	–	–	–	–	3.4	–	–	2.2	–	–
Symmetrospora	–	–	–	–	–	–	3.8	–	–	–	1.2	–
Malassezia	6.3	–	–	–	–	–	1.3	2.3	0.8	14.6	–	–
Filobasidium	–	–	–	–	–	–	–	–	0.4	6.1	–	–
Kondoa	1.0	1.3	–	–	–	–	1.9	1.2	–	–	–	–
Laetiporus	–	–	–	–	–	–	–	0.8	–	1.1	–	–
Rhodotorula	–	–	–	–	–	–	–	0.6	0.7	2.2	–	–
Peniophora	0.6	–	–	–	–	–	–	–	–	0.7	–	2.4
Ganoderma	0.5	–	–	–	–	–	0.5	1.2	–	–	–	4.2
Fomitopsis	–	–	–	–	–	–	0.1	1.5	–	2.6	–	–

include lichenized fungi. Fungi from orders *Chaetothyriales*, *Dothideales*, *Capnodiales*, *Helotiales* and *Pleosporales* were dominant. The order *Chaetothyriales* reached up to 98% of DNA in all studied samples. The order *Dothideales* was second in abundance (up to 40% in all samples).

At the genus level 190 taxa were identified. Most of them (114) were only single findings in only one sample. The absolute dominant in all samples was the genus *Knufia* (up to 85% of the total abundance). In most samples the genus *Aureobasidium* reach to 21% of the abundance. Main genera of fungi with a large and more or less significant proportion are presented in Table 3.

The approach of dividing fungal diversity into groups was used in the same way as for the cultivated microfungi. The genera of *Ascomycota* were divided into seven groups.

The first group MCFs is the most numerous in terms of the number of species and the largest in terms of abundance. This group includes six genera: *Knufia*, *Celosporium*, *Vermiconia*, *Neophaeococcomyces*, *Conio-*

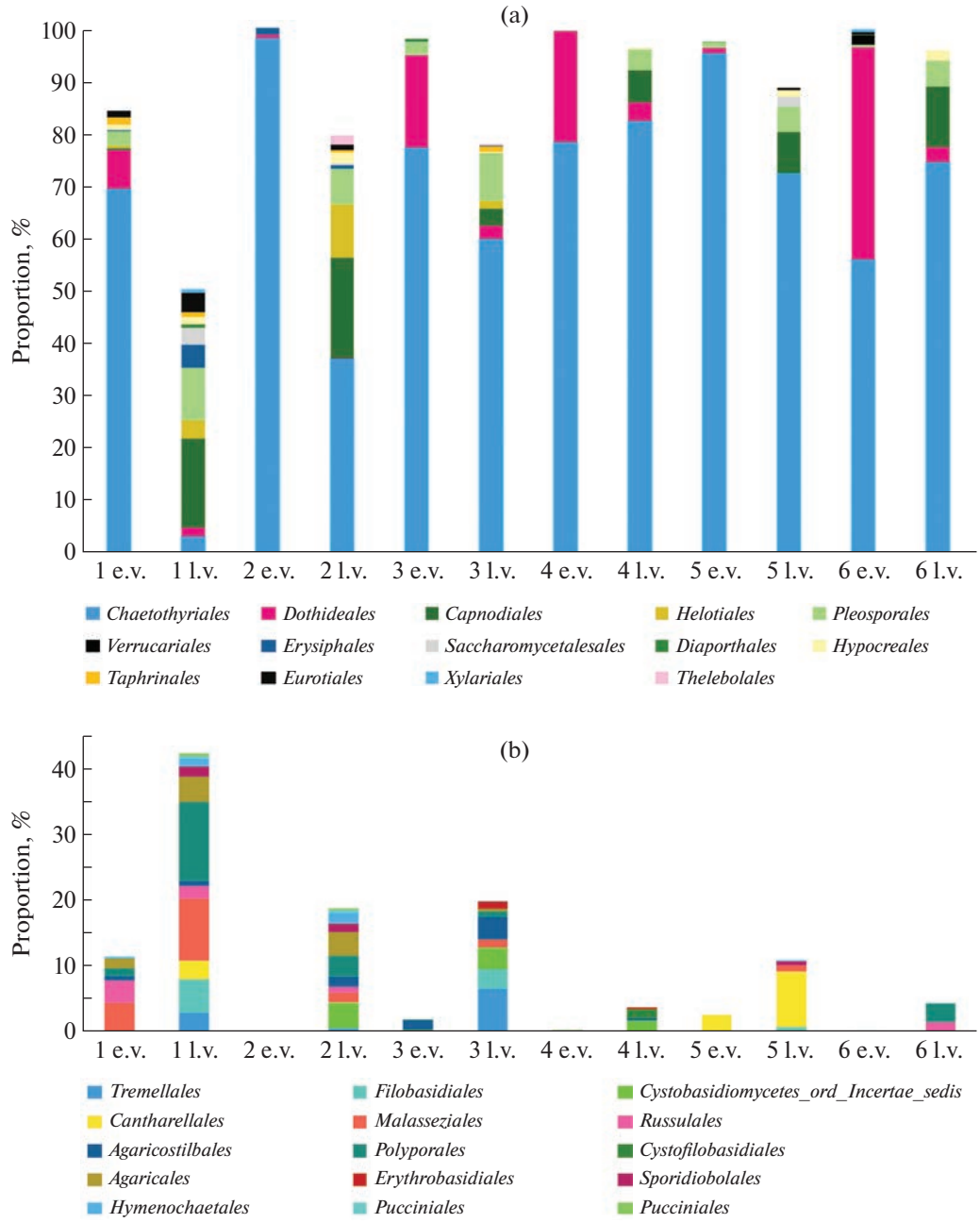

Fig. 4. Abundance of the main orders of fungi in SABs according to metagenomic analysis: a – *Ascomycota*, b – *Basidiomycota*. Samples 1, 2 – SAB1; 3, 4 – SAB2, 5, 6 – SAB3; 1, 3, 5 – marble, 2, 4, 6 – granite.

sporium, *Bradymyces*. The second most abundant group is the fungi with *Aureobasidium*-like morphology: *Aureobasidium*, *Rhinocladiella*, *Saccothecium*, *Exophiala*. The third abundant group is the *Cladosporium* complex, including *Cladosporium*, *Capronia* and *Trichomerium*. The fourth group is presented by fungi of the *Alternaria* complex (large-spore microfungi): *Paracamarosporium*, *Alternaria* and *Stemphylium*. Fungi of this group were found in all samples, but their abundance did not exceed 4%. Fungi of these four groups were found in almost all samples, their frequency of occurrence tended to 100%.

Representatives of subsequent groups met much less frequently. The frequency of their occurrence did not exceed 60%, and the abundance did not exceed 5%. The yeast fungi from *Saccharomycetaceae* (fifth group) are usually associated with the presence of readily available organic substrates (sugars): *Candida*, *Debaryomyces*, *Hanseniaspora*. The sixth group is fungi associated with plants – *Erysiphe*, *Taphrina*, *Podosphaera*, *Cytospora*, *Phaeosphaeria*, *Pseudoophiobolus*. This taxonomically combined group unites fungi that live both on living plants (biotrophs and hemibiotrophs) and on plant substrates of various origins (saprotrophs). The

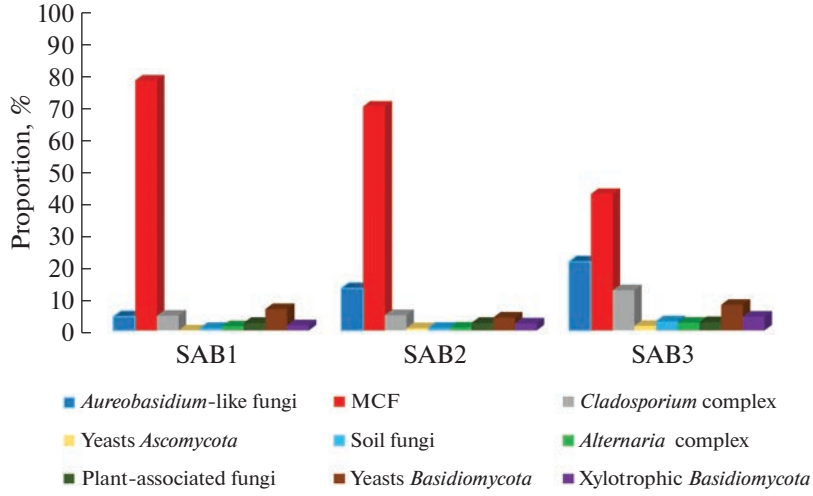

Fig. 5. Shares of some groups of fungi in different SABs (metagenomic data).

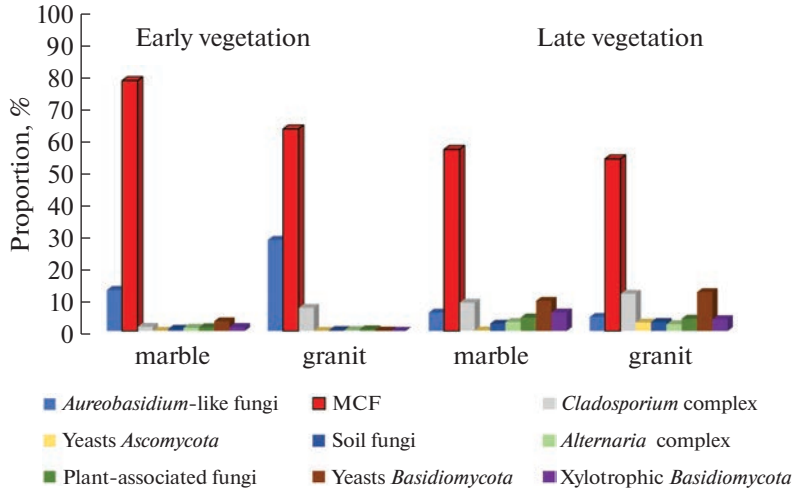

Fig. 6. Shares of some groups of fungi in SABs from various rocks at different seasons (metagenomic data).

seventh group is other fungi associated with the soil and easily spread through the air: *Penicillium*, *Aspergillus*, *Epicoccum*, and *Tolypocladium*.

The genera of *Basidiomycota* can be divided into two main groups. The first of them includes 13 genera of basidiomycete yeast with different ecology: *Vishniacozyma*, *Naganishia*, *Symmetrospora*, *Malassezia*, *Kondoa*, *Buckleyzyma*, *Filobasidium*, *Rhodotorula*, *Udeniomyces*, *Bensingtonia*, *Kurtzmanomyces*, *Tausonia*, and *Erythrobasidium*. The second group includes 14 genera of wood-rotting fungi: *Baltazaria*, *Laetiporus*, *Phlebia*, *Heterobasidion*, *Peniophora*, *Ganoderma*, *Pleurotus*, *Sistotrema*, *Hyphodontia*, *Botryobasidium*, *Bjerkandera*, *Fomes*, *Fomitopsis*, *Oxyporus*.

The main dominant genus *Knufia* is represented by three species: *Knufia karalitana*, *K. endospora* and *K. peltigerae*. The most abundant species among them is *K. karalitana*. The genus *Aureobasidium* was the second in metagenome and included *Aureobasidium leucospermi* and *A. pullulans*.

Comparison of fungi by types of SABs showed that the dominant MCF complex and mainly the genus *Knufia* are most abundant in SAB1 and SAB2 (Figure 5). The abundance of *Aureobasidium*-like fungi was the lowest in SAB1in contrast to SAB2 and SAB3.

The seasonal distribution of fungi shows that the proportion of *Basidiomycota* increased in autumn (Fig. 4b). The abundance of *Ascomycota* fluctuated in different ways. The orders *Capnodiales* and *Pleosporales* dominate in autumn. The order *Dothideales* dominates in spring (Fig. 4a). At the genera level, seasonal differences varied for different types of SABs. The main trends were as follows: the abundance of *Aureobasidium*-like fungi and MCF increased in the spring and decreased in autumn (Fig. 6). The shares of other groups increase in autumn. Differences between marble and granite are minimal (Fig. 6).

DISCUSSION

SABs on the stone monuments surface in Saint Petersburg are abundantly inhabited by fungi, the detection of which depends on the methodological approach. According to culture-based studies, a total of 20 species of fungi have been identified. These data are consistent with the previously obtained results of the microfungi diversity on monuments in the Museum Necropolis of Saint Petersburg which has been carried out since 1999 (Vlasov et al., 2002; Sazanova et al., 2020). The overall species diversity was significantly higher than in one vegetative period described in this study that is obviously associated with a much larger number of samples taken from different monuments covered with biofilms. At the same time, the general trends in the formation of mycobiota at the monuments of the Museum Necropolises are the same. The super-dominant in terms of frequency was the fungus *Aureobasidium pullulans*, which was also confirmed in this study. The dominant species included *Alternaria alternata* and *Cladosporium cladosporioides* (over 50% frequency).

The results of metagenomics analysis made it possible to reveal a significantly greater diversity of microfungi at different taxonomic levels. At the genus level, the number of identified fungi was almost 15 times higher (190 genera).

Obviously, the identified fungi include both the true inhabitants of the stone and fungi, the spores of which fell on the surface of the stone from the environment. Therefore, in addition to the taxonomic diversity of fungi, it is important, in our opinion, to take into account the ecological and morphological features that formed the basis for dividing fungi into groups. On the basis of cultural data 5 groups were identified, and on the basis of metagenomic data, nine groups were identified, two of which formed by basidiomycete fungi.

Both methodological approaches (cultivation and metagenomics) clearly indicate that the complex of dominant fungi in SABs on marble and granite in Saint Petersburg is formed by dark-colored microfungi. The dark-colored microfungi from the genera *Cladosporium*, *Aureobasidium*, *Alternaria*, *Exophiala* and MCF also were often found in urban environment on the surface of calcarenite, granite, limestone, marble, plaster, sandstone, tufa in various regions of the world, mainly in Mediterranean and temperate climate (Cappitelli et al., 2007; Suihko et al., 2007; Farooq et al., 2015; Ortega-Morales et al., 2016; Trovão et al., 2019; Mang et al., 2020; Santo et al., 2021; Paiva et al., 2022).

According to metagenomics studies the fungi from orders *Chaetothyriales*, *Dothideales*, *Capnodiales*, *Helotiales* and *Pleosporales* are dominant. The absolute dominant in occurrence in all samples was *Knufia karalitana*, which was not identified by cultivation methods. Isolation of MCF using conventional culture methods is notoriously difficult, and it requires special methodological approaches (Wollenzien et al., 1995; Vlasov et al., 2002). In addition, identification of isolates is often not possible based on culture and microscopic morphology, and molecular diagnostics are required (Isola et al., 2016).

According to the results of culture-dependent analysis the abundance of MCF reached 13%. We assume the presence of the genera *Coniosporium* spp. and *Knufia* spp. in MCFs complex. Species of this genera form similar morphological structures and their exact identification is possible only with the use of molecular analysis (Isola et al., 2016; De Leo et al., 2019). There is evidence that *Knufia* actively penetrates into freshly exposed stone surfaces regardless of their porosity due to the formation of iron-chelating molecules (De Leo et al., 2022). The species *K. karalitana* was first isolated and described on monuments in Italy, but it is a less common species on stone compared to *K. petricola* and *K. marmoricola*. Diversity of MCF on Saint Petersburg stone monuments is significantly lower than in Mediterranean area.

In our research fungi of different ecological groups (extremophiles, phytopathogens, wood-rotting fungi, soil-inhabiting, yeasts, and even litter saprotrophs) are found on the monuments in Saint Petersburg. Typical soil fungi (species of the genera *Aspergillus*, *Penicillium*, *Trichoderma*, *Botrytis*, *Fusarium*) as well as microfungi connected with plant substrates were found in all types of SAB. These fungi as well as basidiomycete from *Russulales*, *Agaricales*, and *Polyporales* are occasional for lithobiotic communities and occur due to the retention of spores in biofilms on the stone surface. It is interesting to note that basidiomycete fungi increase in abundance in autumn that can be connected with the fruiting bodies formation in this period. At the same time, the abundance of *Aureobasidium*-like fungi and MCF was more observed in the spring.

Biodiversity of fungi on stone surface also depends on the characteristics of SABs. The maximum biodiversity of microfungi was noted in biofilms with lichens domination that was shown by cultural and metagenomics data. This can be explained by the fact that spores of fungi deposited from the atmosphere are more retained in such type of SAB.

The obtained data generally show that the formation of mycobiota on stone monuments in the urban environment is a multifactorial process. The most objective characteristics of the mycobiota can be obtained using a complex of cultural and molecular genetics methods, taking into account the characteristics of biofilms, the nearest environment and the influence of the season.

CONCLUSION

The mycobiota of sculptural monuments in the historical center of Saint Petersburg has a complex structure. There are different complexes of species that play a decisive role in the stone monuments colonization (MCF, *Aureobasidium*-like fungi, *Cladosporium* com-

plex, *Alternaria* complex). Fungi associated with plant substrates and soil are also constantly present in the SAB on the surface of the monuments. The use of two methodological approaches indicates the expediency of combining cultural and molecular genetic methods, which make it possible to obtain the most diverse picture of the formation of lithobiotic communities. The season has a key importance for the abundance and diversity of microfungi on stone surface. Obviously, the type of rock has a minimal importance for the fungal diversity on stone monument. At the same time, the SAB type seems to significantly affect the formation of mycobiota due to the different ability to accumulate fungal propagules from the environment. Results of the study can be used in the development of methods for monuments protection from biodeterioration.

This research was supported by the Russian Science Foundation (№ 21-74-00031). The authors are grateful to Beagle LLC for their cooperation in performing metagenomic analysis.

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Особенности формирования микобиоты на каменных памятниках Санкт-Петербурга по данным метагеномного и культурального исследования

К. В. Сазанова[a,b,c,#], М. С. Зеленская[b,##], О. А. Павлова[d,###], Д. Ю. Власов[a,b,####]

[a]*Ботанический институт им. В.Л. Комарова РАН, Санкт-Петербург, Россия*

[b]*Санкт-Петербургский государственный университет, Санкт-Петербург, Россия*

[c]*Архив Российской академии наук, Санкт-Петербургское отделение, Санкт-Петербург, Россия*

[d]*ООО Бигль, Санкт-Петербург, Россия*

[#]*e-mail: ksazanova@binran.ru*

[##]*e-mail: marsz@yandex.ru*

[###]*e-mail: kmitry.vlasov@mail.ru*

[####]*e-mail: beagle07@bk.ru*

Грибы играют важную роль в колонизации и биоповреждении каменных памятников на открытом воздухе. Это исследование существенно дополняет данные о разнообразии грибов в литобионтных сообществах за счет использования метагеномного анализа. Показано, что микобиота надгробных памятников в историческом центре Санкт-Петербурга имеет сложную структуру. Существуют разные комплексы видов, имеющих различное происхождение и пути попадания в памятники. Комплекс доминирующих грибов в САБ на мраморе и граните Санкт-Петербурга формируют темноокрашенные микромицеты. Абсолютным доминантом во всех выборках был вид *Knufia karalitana* по метагеномным данным и *Aureobasidium pullulans* по культуральным данным. Использование двух методологических подходов указывает на целесообразность сочетания культуральных и молекулярно-генетических методов, позволяющих получить наиболее полную картину формирования литобионтных сообществ. Сезон и тип биопленки имеют ключевое значение для обилия и разнообразия микромицетов на поверхности камня. Показано, что тип породы имеет минимальное значение для разнообразия грибов на каменном памятнике.

Ключевые слова: культуральный и метагеномный методологические подходы, культурное наследие, микроскопические грибы, микробное сообщество, разнообразие грибов, субаэральные биопленки