Thermophilic and thermotolerant microfungi of coal mine spoil tips beyond the Arctic Circle

Microhongos termofílicos y termotolerantes de las escombreras de las minas de carbón más allá del Círculo Ártico

Vadim A. Iliushin[1]* & Irina Y. Kirtsideli[1]

1. Komarov Botanical Institute, Russian Academy of Sciences, Saint Petersburg, Russia

* ilva94@yandex.ru

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Summary

Background and aims: Thermophilic and thermotolerant fungi occupy a variety of ecological niches. The purpose of this article is to show that these microfungi are able to live even in such harsh conditions of the high Arctic beyond the Arctic Circle.

M&M: Rock from coal mine spoil tips were collected and studied using scanning electron microscopy. The method of direct inoculation was employed to isolate microfungi. The isolates were identified using morphological identification and molecular methods. The growth rate of micromycetes was studied depending on temperature.

Results: Eight cultures of microfungi belonging to six species that are able to grow at temperatures of 45 °C and above were isolated from coal mine spoil tips.

Conclusions: The data obtained indicate the presence of thermophilic and thermotolerant microfungi in the spoil tips of coal mines beyond the Arctic Circle. All the fungi belonged to the order Eurotiales. These microfungi are able to exist in the harshest conditions with several simultaneous adverse factors.

Key words

Arctic, coal mine, microfungi, spoil tips, thermophilic, thermotolerant.

Resumen

Introducción y objetivos: Los hongos termófilos y termotolerantes ocupan una variedad de nichos ecológicos. El objetivo de este artículo es mostrar que estos hongos microscópicos son capaces de vivir incluso en las duras condiciones del alto Ártico más allá del Círculo Polar Ártico.

M&M: Se recogieron y estudiaron rocas de los restos de las minas de carbón mediante microscopía electrónica de barrido. Se empleó el método de inoculación directa para aislar microhongos. Los aislamientos fueron caracterizados mediante identificación morfológica y métodos moleculares. La tasa de crecimiento de los micromicetos se estudió en función de la temperatura.

Resultados: Se lograron ocho cultivos de hongos microscópicos pertenecientes a seis especies que pueden crecer a temperaturas de 45 °C y superiores de los escombros de las minas de carbón.

Conclusiones: Los datos obtenidos indican la presencia de hongos microscópicos termófilos y termotolerantes en los vertederos de minas de carbón más allá del Círculo Polar Ártico. Todos los hongos pertenecían al orden Eurotiales. Estos hongos microscópicos pueden existir en las condiciones más duras con varios factores adversos simultáneos.

Palabras clave

Ártico, microhongos, mina de carbón, termófilo, termotolerante, vertedero de carbón.

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Introduction

Fungi are capable of existing at constantly high temperatures. These fungi can be classified as thermophilic or thermotolerant, depending on the optimal growth temperature. Thus, fungi are considered thermophiles if the optimal growth temperature is 45 °C or higher (Mouchacca, 2000). Thermophiles are considered as a special case of extremophiles.

Thermotolerant and thermophilic fungi can be isolated from a wide variety of places where the substrate is heated. Examples of such habitats can be compost pits, urban landfills of household and industrial waste - large, moist, isolated accumulations of organic matter, where internal temperatures rise as a result of microbial respiration (thermogenesis) (Zak & Wildman, 2004). Thermophilic and thermotolerant fungi can also be isolated from bird nests (Korniłłowicz-Kowalska & Kitowski, 2013). Thermophilic micromycetes are characteristic of the mycobiota of thermal springs (Pan *et al.*, 2010). Microfungi are ubiquitous in soils where the sun can heat the surface to high temperatures. It is noted that these fungi can make up a significant part of the mycobiota of soils in arid and tropical regions (Mouchacca, 2000). In addition to the listed habitats, thermophilic and thermotolerant micromycetes are the most important part of the mycobiota of coal mine spoil tips (Evans, 1971; Tansey & Brook, 1978; Bilaj, 1985).

The first studies of the microbiota of coal deposits were carried out in 1928 (Lieske & Hofmann, 1928). The work described a wide range of microorganisms, including micromycetes, living in German mine spoil tips.

The greatest interest in the mycobiota of coal mine spoil tips (waste heaps, slagheaps) is due to the fact that tips can serve as a habitat for thermophilic and thermotolerant species. Strong heating of the insolated surface of the slope (Manakov & Kupriyanov, 2009), as well as oxidative processes occurring in the thickness of the tip, leading to heating (Lewińska & Dyczko, 2016) or even spontaneous combustion (Querol *et al.*, 2008), create unfavorable conditions for most types of fungi. However, such an extreme habitat is suitable for the formation of thermophilic mycobiota.

Thermophilic fungi were found on the surface of rocks during coal mining in areas with high surface temperatures caused by combustion inside the tip, and in areas with normal surface temperatures. In rock samples from areas with elevated temperatures, significantly more thermophilic micromycetes were found than from areas with normal temperatures (Tansey & Brook, 1978).

The rock tips of the Chandameta, Parasiya and Newton Chikli coal mines (Madhya Pradesh, India) are characterized by spontaneous combustion processes, which, together with the arid climate, makes these habitats especially extreme (Salar, 2018). As a result of the study of the mycobiota of these tips, 14 species of microfungi belonging to the genera *Absidia* Tiegh., *Achaetomium* J.N. Rai, J.P. Tewari & Mukerji, *Aspergillus* P. Micheli ex Haller, *Emericella* Berk., *Humicola* Traaen, *Penicillium* Link, *Rhizopus* Ehrenb., *Sporotrichum* Link, *Thermoascus* Miehe, *Thermomyces* Tsikl., *Thielavia* Zopf, and *Torula* Pers. were discovered (Johri & Thakre, 1975; Thakre & Johri, 1976). Two new yeast endemic species, *Debaryomyces singareniensis* Saluja & G.S. Prasad and *Torulaspora indica* Saluja, Yelchuri, Sohal, Bhagat, Paramjit & G.S. Prasad, have been identified from the rock of coal mine spoil tips in another region of India, Andhra Pradesh (Saluja & Prasad, 2007; Saluja *et al.*, 2012).

The biodiversity of technogenic spoil tips has been studied across industrial regions in various climatic zones. However, the degree of knowledge of the mycobiota of various coal mining areas is very heterogeneous. Thus, almost all the few studies are devoted to the study of rock tips of coal mines and quarries in the temperate zone of Europe (Evans, 1971; Ulfig & Korcz, 1995; Detheridge *et al.*, 2018). Studies of coal tips in the Arctic are few and focus primarily on Svalbard (Iliushin, 2020; Iliushin *et al.*, 2022a), the Komi Republic (Khabibullina *et al.*, 2015; Iliushin & Kirtsideli, 2021), and the Magadan region (Iliushin *et al.*, 2022b).

In this work, for the first time, thermophilic and thermotolerant strains of microfungi were screened in the Arctic Circle.

Materials and Methods

Sampling

Samples for the mycological research were taken from spoil tips of the coal mine "Nos. 1-5" near

Barentsburg village (78° 03′ 51″ N, 14° 11′ 09″ E) on the Svalbard (Norway) from July to August 2018; from spoil tips of the Komsomolskaya coal mine and the spoil tip of the Pechora Central Processing Plant near the city of Vorkuta (67° 30′ N, 64° 02′ E) Komi Republic (Russia) at the end of July 2019 and from spoil tips of the coal mine “Tal-Yuryakh” near the town of Susuman (62° 47′ N, 148° 09′ E) Magadan region (Russia) in august 2020 (Fig. 1). The samples were obtained from

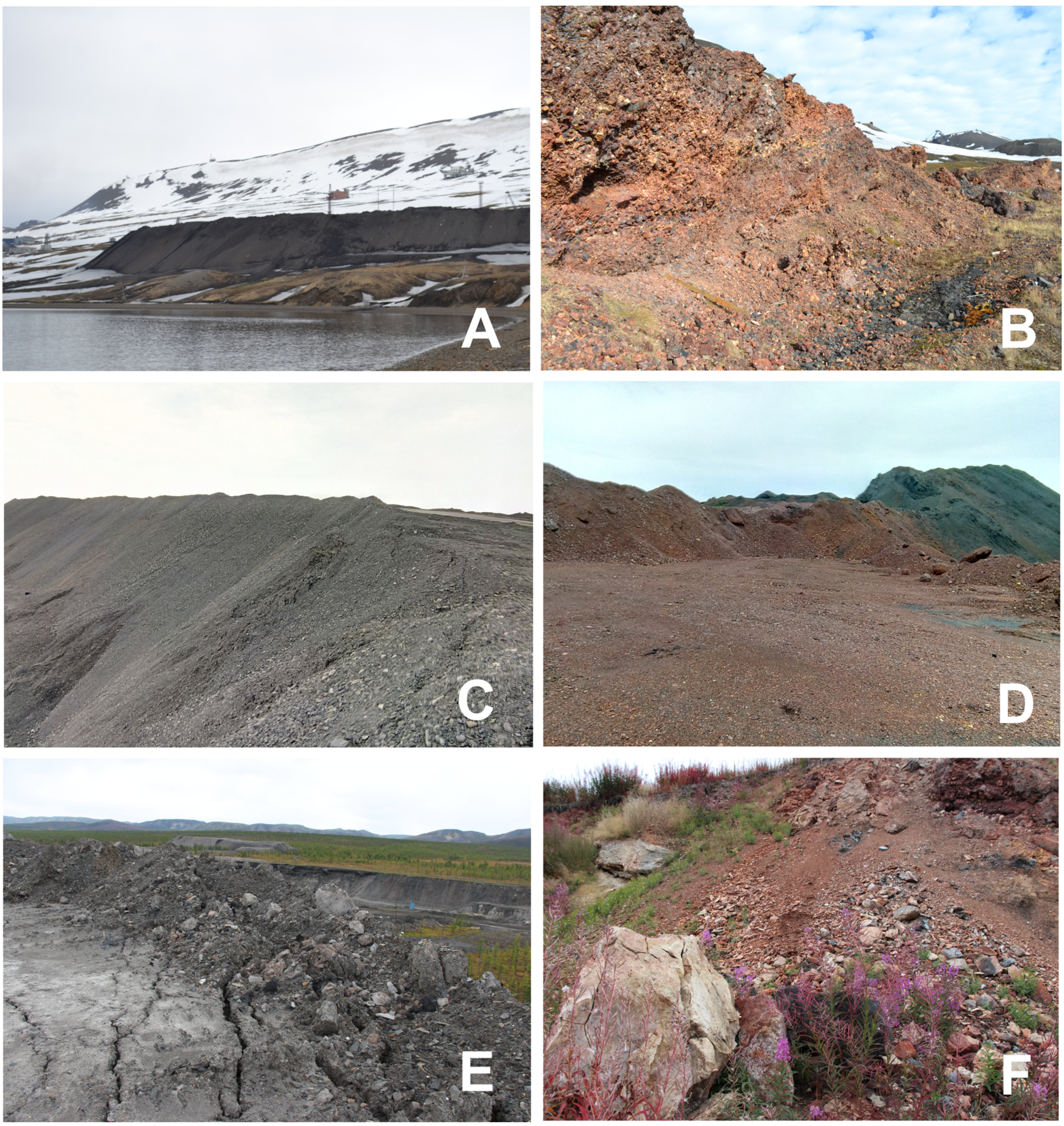

Fig. 1. Sampling locations. **A**: Formed spoil tip from Svalbard. **B**: Spoil tip with burnt rock from Svalbard. **C**: Formed spoil tip from Komi Republic. **D**: Spoil tip with burnt rock from Komi Republic. **E**: Formed spoil tip from Magadan region. **F**: Spoil tip with burnt rock from Magadan region.

formed (exploited) spoil tips and spoil tips with burnt rock of the coal mines. Burnt rocks are formed during high-temperature transformations of primary rocks in an oxygen atmosphere (Nenakhova, 1989). Thus, the mycobiota of two types of ecosystems associated with coal mining, located in three natural zones (arctic tundra, southern tundra, and forest-tundra) has been studied. Geobotanical descriptions were performed on the selected sites, and average samples of soil and spoil tip rock were selected (Table 1). Samples of rock for mycological analysis were collected in individual sterile 50-milliliter plastic tubes. All samples were stored at 4 °C.

Burnt rocks are characteristic of man-made ecosystems, but sometimes they are formed as a result of natural processes. The temperature may locally rise in the thickness of the rock tip due to oxidative processes. At the same time, due to the high concentration of sulfur, changes in the level of moisture, as well as a large amount of residual coal, spontaneous combustion can occur (Querol *et al*., 2008; Bragina, 2013). The composition and properties of burnt rocks are variable and depend on the composition of the source rocks and the degree of their firing (Nenakhova, 1989). External changes in the tips are manifested in the reddening of the source rocks. It should also be noted that due to the combustion processes in tips, pedogenesis can slow down or even stop (Bragina, 2013).

Isolation, incubation and identification

The method of direct inoculation of small fragments of substrate was employed to isolate microfungi (Dudka *et al*., 1982). Fungi were cultivated on solid media Czapek agar (CZ) and Sabouraud agar at 45 °C (Raper & Thom, 1949; Kashkin *et al*., 1979). Chloramphenicol (100 mg/L) was added to the culture medium to suppress the growth of bacteria.

The isolates of each species were initially identified by morphological identification using the identification handbooks, manuals and articles after their isolation in pure culture (Egorova, 1986; Domsch *et al*., 2007; Samson *et al.,* 2010; Houbraken *et al.,* 2012; Hubka *et al.,* 2013). For micromorphological examination, microscopy by Carl Zeiss AxioImager A1 was employed.

The isolates were also identified by molecular methods. DNA was extracted by using a Diamond DNA Plant kit (ABT, Russia, Barnaul) according to the manufacturer's instructions. Internal transcribed spacer rDNA region (ITS1-5.8S-ITS2) amplified using the PCR-primers ITS1 (5′-TCC-GTA-GGT-GAA-CCT-TGC-GG-3′) and ITS4 (5′-TCC-TCC-GCT-TAT-TGA-TAT-GC-3’) and applied as a phylogenetic marker (White *et al*., 1990). For amplification of the partial ribosomal polymerase II second largest subunit (RPB2) primers 5F_Eur (5′-GAY-GAY-CGK-GAY-CAY-TTC-GG-3′) and 7CR_Eur (5′- CCC-ATR-GCY-TGY-TTRCCC- AT-3′) were used (Houbraken & Samson, 2011). The D1/D2 region of 28S rDNA (LSU) was amplified using the PCR-primers NL-1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL-4 (5′- GGTCCGTGTTTCAAGACGG -3′) (O’Donnell, 1993). The agarose gel electrophoretic method was applied to separate the DNA in the samples after amplification. Sequencing of the obtained DNA fragments was carried out by the ABI 3500 (Thermo Fisher Scientific, USA) using the Sanger method. Sequences were inspected using

Table 1. Sampling locations.

Sampling locations	Type of spoil tip	Name of mine or quarry	Coordinates	Projective coverage
Svalbard	formed (exploited) spoil tip	Mine No. 1-5	78°02’57” N, 14°13’18.1” E	0%
	spoil tip with burnt rock	Mine No. 1-5	78°02’04” N, 14°18’08” E	0%
Komi Republic	formed (exploited) spoil tip	spoil tip of the Pechora Central Processing Plant	67°30’18” N, 63°40’44” E	0%
	spoil tip with burnt rock	Komsomolskaya	67°29’11” N, 63°46’59” E	0%
Magadan region	formed (exploited) spoil tip	Tal-Yuryakh	63°20’10” N, 146°37’10” E	0%
	spoil tip with burnt rock	Tal-Yuryakh	63°17’10” N, 146°40’03” E	40%

BioEdit version 7.1.9. The obtained sequences were submitted to the NCBI GenBank and compared to the available sequences database by using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The criteria were used to interpret the sequences: the genus and species were accepted for sequence identities ≥ 98%; only the genus was accepted between 95% and 97% (Godinho *et al*., 2013).

Study of growth rate depending on temperature

The growth rate of thermophilic and thermotolerant micromycetes was studied depending on temperature on Czapek agar (CZ) and Sabouraud medium. Cultivation was carried out in thermostats and refrigerators at temperatures of 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, 35 °C, 45 °C, 50 °C, 55 °C, 60 °C, 62 °C, 65 °C. If necessary, micromycetes were cultivated at intermediate temperature values. Cultivation was carried out in the dark for 7-21 days (depending on the strain and temperature).

Analysis of the rock of coal mine spoil tips

The composition of the samples was characterized using scanning electron microscopy and electron probe microanalysis using a desktop scanning electron microscope-microanalyzer TM 3000 (HITACHI, Japan) equipped with an OXFORD energy dispersion microanalysis console at the St. Petersburg State University Microscopy and Microanalysis Resource Center, as well as X-ray fluorescence analysis using a portable DELTA XRF analyzer (Olympus, Japan).

Result

As a result of the study of the spoil tips of coal mines of Svalbard, the Komi Republic and the Magadan region, eight cultures of microfungi belonging to six species that are able to grow at temperatures of 45 °C and above were isolated (Table 2). The observed macro- and micromorphology (Figs. 2-3 respectively) of the obtained isolates was consistent with the micro- and macromorphology of the corresponding species (Egorova, 1986; Domsch *et al.*, 2007; Samson *et al.,* 2010; Houbraken *et al.,* 2012; Hubka *et al.,* 2013). BLAST analysis of the ITS region, LSU or RPB2 showed 99-100% similarity of the isolates and corresponding species. Therefore, molecular and morphological data made it possible to conclude that the obtained isolates belongs to the species *Aspergillus waksmanii* Hubka, S.W. Peterson, Frisvad & M. Kolařík, *Aspergillus spinosus* Kozak, *Rasamsonia cylindrospora* (G. Sm.) Houbraken & Frisvad, *Rasamsonia byssochlamydoides* (Stolk & Samson) Houbraken & Frisvad, *Rasamsonia emersonii* (Stolk) Houbraken & Frisvad, and *Thermoascus aurantiacus* Miehe.

Four species (five strains) of fungi resistant to the temperature factor were isolated from the formed spoil tip of the Svalbard coal mine (unburned rock). It should be noted that thermotolerant and thermophilic micromycetes were not isolated from the tips of unburned rock of the Komi Republic and the Magadan region.

Table 2. Culturable microfungi of coal mine spoil tips.

Strain	Species	Habitat	GenBank accession numbers	Phylogenetic marker
IVA-T1	*Rasamsonia emersonii*	formed spoil tip, Svalbard	**OP919555**	ITS
			OP919563	LSU
IVA-T2	*Aspergillus waksmanii*	spoil tip with burnt rock, Svalbard	**OP889566**	ITS
IVA-T4	*Rasamsonia byssochlamydoides*	formed spoil tip, Svalbard	**PP182127**	ITS
IVA-T6	*Aspergillus spinosus*	spoil tip with burnt rock, Komi Republic	**OP889565**	ITS
IVA-T7	*Rasamsonia cylindrospora*	spoil tip with burnt rock, Svalbard	**OP889574**	ITS
IVA-T11	*Thermoascus aurantiacus*	formed spoil tip, Svalbard	**PP216935**	RPB2
IVA-T14	*Aspergillus waksmanii*	formed spoil tip, Svalbard	**OP919556**	ITS
IVA-T16	*Aspergillus waksmanii*	formed spoil tip, Svalbard	**OP919564**	LSU

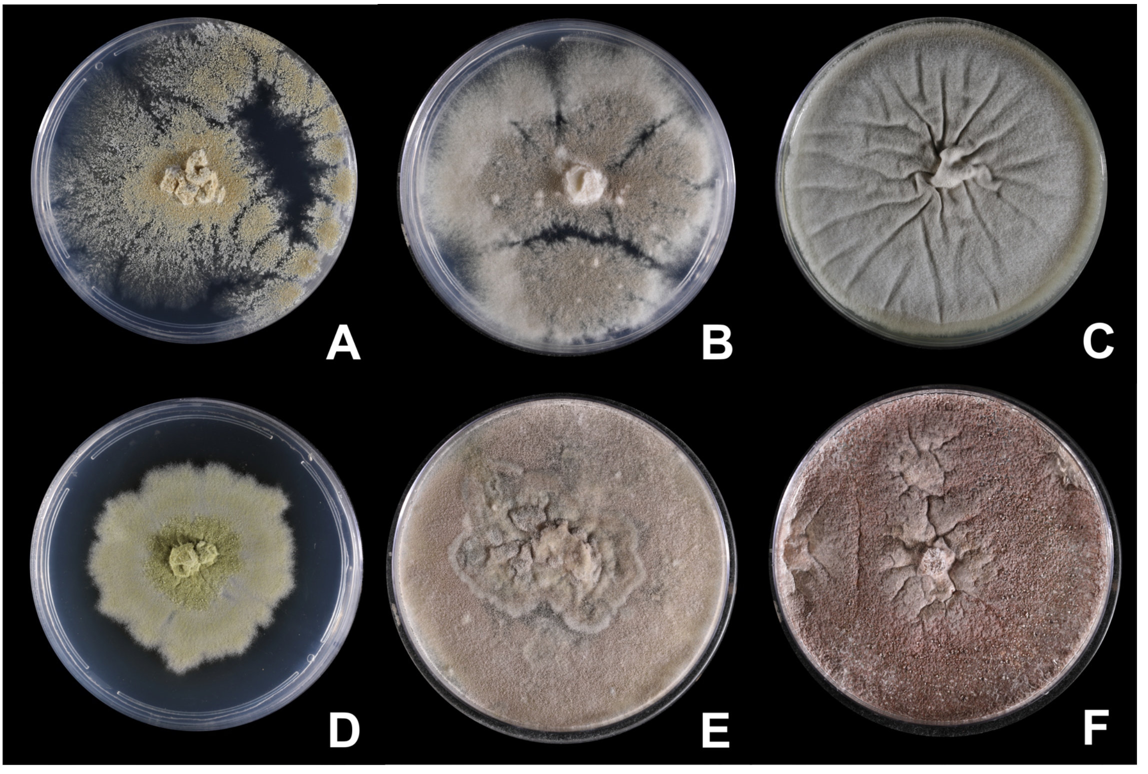

Fig. 2. Colony of microfungi of coal mine spoil tips. **A**: IVA-T1 *Rasamsonia emersonii.* **B**: IVA-T2 *Aspergillus waksmanii*. **C**: IVA-T4 *Rasamsonia byssochlamydoides.* **D**: IVA-T7 *Rasamsonia cylindrospora*. **E**: IVA-T6 *Aspergillus spinosus*. **F**: IVA-T11 *Thermoascus aurantiacus*.

Two isolates of *Aspergillus waksmanii*, IVA-T14 and IVA-T16, and isolate IVA-T4 *Rasamsonia byssochlamydoides* belonged to thermotolerant species. Thus, the temperature optimum was 35-45 °C for these species, but almost no growth was observed at 50 °C. The real thermophiles included two isolates, IVA-T1 *Rasamsonia emersonii* and IVA-T11 *Thermoascus aurantiacus*. The former was characterized by a relatively narrow temperature optimum of 45-50 °C, as well as a high maximum growth temperature of 60 °C. On the contrary, the latter isolate exhibited a wide temperature optimum of 40-60 °C and an extremely high maximum growth temperature of 62 °C. Figure 4 shows the growth curves of isolates obtained from coal tips.

Three thermotolerant fungi were isolated from the tips of burnt rock of Svalbard (IVA-T2, IVA-T7) and the Komi Republic (IVA-T6). Thermotolerant and thermophilic micromycetes have not been isolated from the tips of burnt rock in the Magadan region. Two isolates belonged to thermotolerant species of the genus *Aspergillus*. For IVA-T2 *Aspergillus waksmanii*, the temperature optimum was 35-45 °C, no growth was observed at 50 °C. IVA-T6 *Aspergillus spinosus* was characterized by a lower temperature optimum of 30-40 °C, at the same time, a slight increase was observed at 50 °C. For the third species, *Rasamsonia cylindrospora* (isolate IVA-T7), the optimum and maximum growth temperature were higher, at 40-50 °C and 60 °C, respectively, which with a small assumption, allows this species to be classified as thermophilic. Figure 5 shows the growth curves of isolates obtained from coal tips of burnt rocks.

In this study, 87.5% of the isolates were obtained from rock samples collected from the spoil tips of Svalbard, while only 12.5% originated from

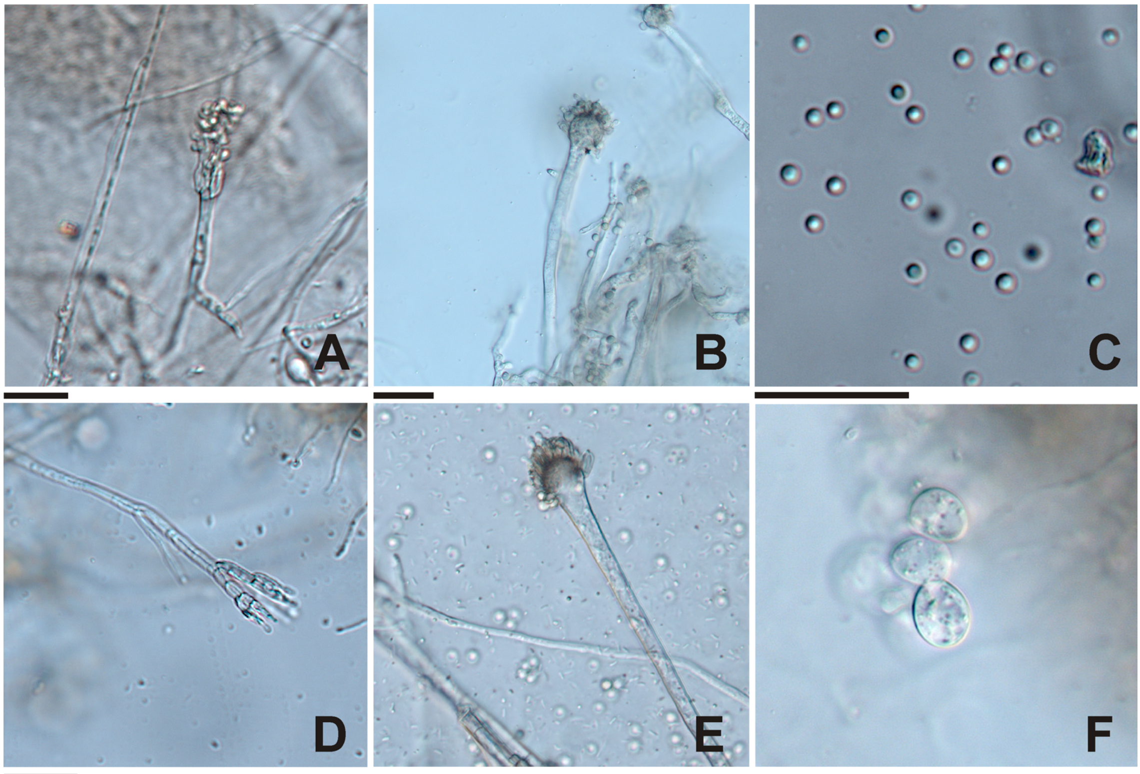

Fig. 3. Micromorphological characters of microfungi of coal mine spoil tips. **A**: Conidiophore of IVA-T1 *Rasamsonia emersonii.* **B**: Conidiophores of IVA-T2 *Aspergillus waksmanii*. **C**: Conidia of IVA-T4 *Rasamsonia byssochlamydoides*. **D**: Conidiophore of IVA-T7 *Rasamsonia cylindrospora*. **E**: Conidiophore of IVA-T6 *Aspergillus spinosus*. **F**: Ascospores of IVA-T11 *Thermoascus aurantiacus*. Scale= 20 μm.

the Komi Republic. Notably, thermophilic and thermotolerant fungi were not isolated from the tips of the Magadan region.

Microphotographs and their corresponding energy dispersive X-ray spectra of rocks are presented in Fig. 6. The elemental composition is presented in Table 3.

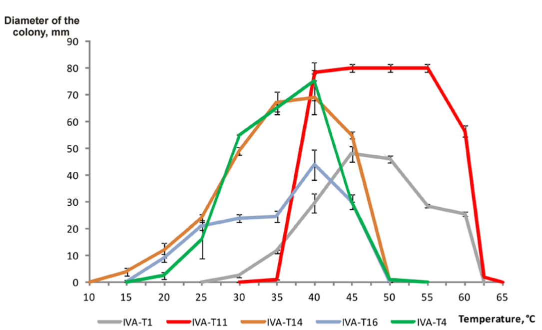

Fig. 4. Growth curves of thermophilic and thermotolerant micromycetes isolated from formed (exploited) spoil tips (unburnt rock).

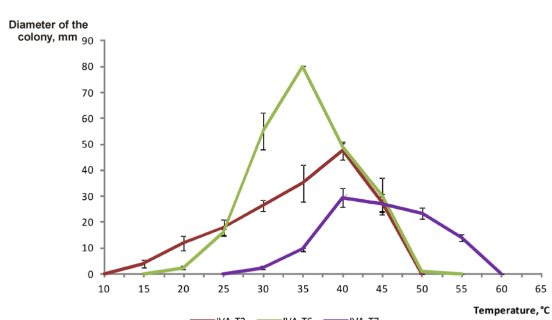

Fig. 5. Growth curves of thermophilic and thermotolerant micromycetes isolated from spoil tips with burnt rock.

The rocks of the studied tips mainly consist of a heterogeneous mixture of clastic rocks: siltstones, mudstones, non-carbonate loams and sandstones. The content of coal particles was detected in the formed tips of Spitsbergen and the Komi Republic. There is porcellanite in burnt rock. The most common minerals were quartz and aluminosilicates (clay minerals, feldspars, micas). It should be noted that the elemental composition turned out to be relatively similar in the tips (Table 3).

Discussion

All isolated thermotolerant and thermophilic species from the technogenic ecosystems studied in this work belonged to three genera within the order Eurotiales: *Aspergillus, Rasamsonia,* and *Thermoascus*. Two species belonged to the genus *Rasamsonia*. All species of this genus are characterized as thermotolerant and thermophilic (Houbraken *et al.,* 2012). Thus, representatives of the genus *Rasamsonia* were found in coal mining sites, residential buildings, peat dumps, compost, and well-warmed soils. Some of them are pathogens causing mycoses in humans; strains are isolated from clinical specimens, including blood culture, bronchial washing and dialysis fluid. Species of the genus *Rasamsonia* are also an important component of aeromycota (Frohlich-Nowoisky *et al.,* 2009).

One of the species identified belonged to the genus *Thermoascus*. This is a small genus represented exclusively by thermophiles (Houbraken *et al.,* 2020). Micromycetes of this genus live in mushroom compost, peat, coal tips, heated soils, sawdust, found in the air, as well as in self-heating hay (Salar, 2018). The highest temperature maximum among fungi belongs to a representative of the genus *Thermoascus* (*T. aurantiacus*). So, this fungus is able to grow at 62 °C (Cooney & Emerson, 1964; Stolk, 1965; Evans, 1971; Domsch *et al.*, 2007; Salar, 2018). This species is very widespread and has been found in Australia, Canada, Egypt, Germany, India, Indonesia, Italy, Japan, Jordan, Netherlands, Russia, South Africa, United Kingdom, and the United States. *T. aurantiacus* lives on various substrates: cacao husks, chaff, coal mine soils,

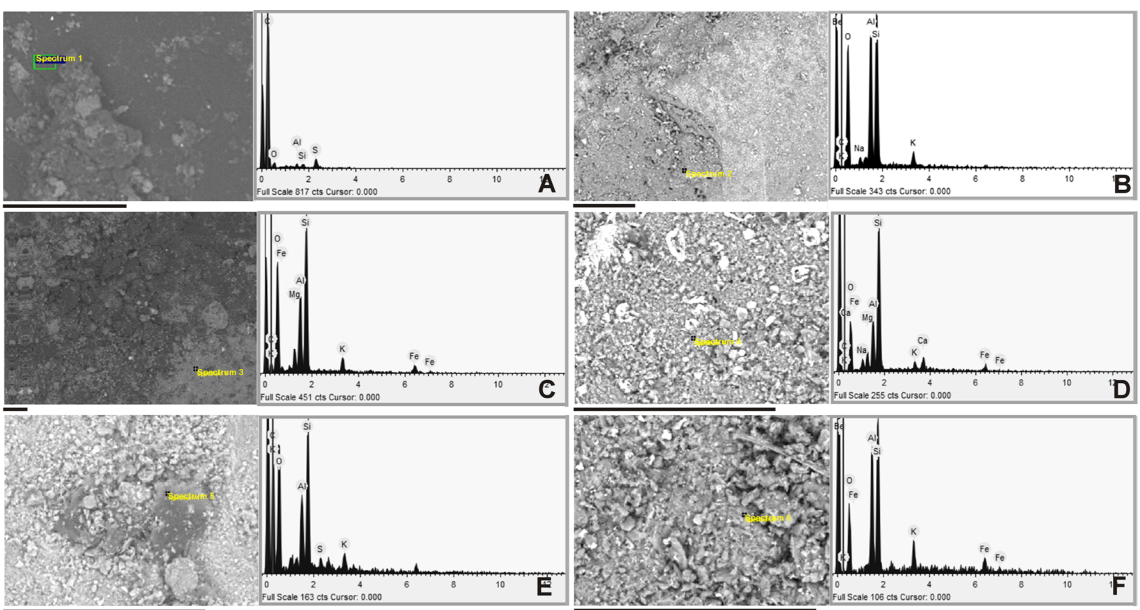

Fig. 6. Microphotographs (SEM) of rock and the corresponding EDS (energy dispersive X-ray spectra). **A**: Spoil tip from Svalbard. **B**: Spoil tip with burnt rock from Svalbard. **C**: Formed spoil tip from Komi Republic. **D**: Spoil tip with burnt rock from Komi Republic. **E**: Formed spoil tip from Magadan region. **F**: Spoil tip with burnt rock from Magadan region. Scale= 100 μm.

Table 3. Elemental composition of spoil tips. All data expressed as a percentage. Abbreviations= <LOD: below the level of detail.

	Formed tip Svalbard	Formed tip Komi Republic	Formed tip Magadan region	Burnt rock tip Svalbard	Burnt rock tip Komi Republic	Burnt rock tip Magadan region
Mg	<0,1	<0,1	<0,1	<0,1	<0,1	<0,1
Al	0,5471	0,9724	1,6947	3,3639	1,3281	1,903
Si	2,3972	6,2361	10,1379	8,8637	7,6233	7,6642
P	0,1	0,1886	0,2249	0,5381	0,225	0,1644
S	1,867	0,4235	<0,01	0,3724	0,1347	1,5838
Cl	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01
K	0,1573	0,3426	0,6473	0,5369	0,3303	0,5197
Ca	0,776	0,805	0,3174	1,61	1,7305	1,9599
Ti	0,0714	0,2431	0,2655	0,5947	0,3008	0,2948
V	<0,007	<0,007	<0,007	0,0194	0,0097	0,007
Cr	<0,003	<0,003	<0,003	0,0053	0,0031	<0,003
Mn	<0,02	0,0416	0,0359	0,0171	0,1531	0,0232
Fe	1,6752	3,7076	2,7565	5,5289	6,2631	2,732
Co	0,0022	0,0056	<0,002	<0,002	<0,002	<0,002
Ni	<0,0009	0,0017	6,00E-04	0,0018	0,0037	1,00E-03
Cu	<0,001	<0,001	<0,001	<0,001	0,0016	<0,001
Zn	<0,0008	0,0015	0,0015	1,00E-03	0,0023	0,0021
As	<0,0007	7,00E-04	4,00E-04	0,0012	0,0015	6,00E-04
Zr	0,0036	0,0037	0,0054	0,0171	0,0056	0,0065
Mo	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Cd	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Sn	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Sb	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
W	<LOD	<LOD	<LOD	<LOD	8,00E-04	<LOD
Pb	<LOD	<LOD	<LOD	<LOD	<LOD	5,00E-04
Bi	3,00E-04	5,00E-04	5,00E-04	0,0015	9,00E-04	8,00E-04
Light elements	92,4027	87,0259	83,9117	78,5271	81,8818	83,1365

heated hay, mushroom compost, peat, sawdust, self-heated wood chips, soil, stored grains, tobacco. Our isolate IVA-T11 *T. aurantiacus* from Svalbard is capable of growing at the maximum temperature recorded for fungi: 62 °C.

Four isolates within the thermotolerant specimens found belonged to two species of the genus *Aspergillus* (*A. spinosus* and *A. waksmanii*). It is a widespread and numerous genus, its representatives occupy many ecological niches and are found in a wide variety of habitats, including extreme ecosystems where elevated temperatures are observed. Thus, fungi of the genus *Aspergillus* live in all the above habitats where the species of the genera *Thermoascus* and *Rasamsonia* are found, and they have also been found in thermal springs, landfills, and bird nests. Fungi of the genus *Aspergillus* are an important component of the mycobiota of deserts (Sterflinger *et al.,* 2012; Sklenar *et al.,* 2017). However, there are no true thermophilic species among this genus, and only thermotolerant ones are found in extreme ecosystems (optimum < 45°C) (Kozakiewicz & Smith, 1994). Most thermotolerant species belong to the Fumigati section. Thus, *Aspergillus fumigatus* is one of the most common species in the rock of coal dumps in the USA, both on the surface of heated areas of tips and at depths up to 30 cm (Tansey & Brook, 1978). All the thermotolerant *Aspergillus* species found in this study belonged to the Fumigati section. One of these species was *A. sibiricus*, which was isolated from a coal mine in Altai (Russia) (Iliushin, 2022). It should also be noted that many species of the Fumigati section can be tolerant of other factors besides high temperature. In particular, *A. fumigatus* and *A. sibiricus* are able to grow at low pH values (2-3) and are acid tolerant species. Tolerance to various environmental factors (elevated temperatures and low pH of the medium) allows micromycetes to exist in such an extreme ecosystem as a burning coal quarry and spoil tips with burnt rocks.

Previous studies have shown that coal mine spoil tips are characterized by low species diversity (Khabibullina *et al*., 2015; Iliushin & Kirtsideli, 2021; Iliushin *et al.,* 2022a, b). At the same time, there is not always a clear pattern of an increase in the number of species to southern latitudes. Probably, the number of species is influenced by the factor of the area of disturbed territories. The main source of the introduction of propagules into technogenic ecosystems is aeromycota (Kirtsideli *et al.,* 2011). Consequently, the larger the area disturbed and the farther the tip is located from natural communities, the less likely it is that viable propagules will be picked up by the air flow and transferred to technogenic ecosystems. On the other hand, besides air transport, invasion of microfungi may occur through other means, with anthropogenic and ornithogenic factors likely playing a relatively significant role (Zhu *et al*., 2011; Kirtsideli *et al*., 2020; Sacramento *et al*., 2023). In particular, on Spitsbergen, where there are many bird colonies, one of the main ones will be the ornithogenic factor. Another important condition is the composition of the rocks of tips. On average, spoil tips with burnt rock had fewer species.

The data obtained indicate the existence of thermophilic and thermotolerant microfungi thriving within the spoil tips of coal mines, including in a substrate with severely degraded conditions such as tips of burnt rock. Probably, the presence of thermotolerant microfungi depends on the ornithogenic factor. Therefore, the largest numbers of isolates were found in the tips on Svalbard and not a single one in the Magadan region. All the microfungi isolated in this work were identified as belonging to the order Eurotiales. This discovery underscores the presence of thermotolerant and thermophilic fungi in the challenging environment of the Arctic Circle.

Authors Contributions

IYK was involved in sampling, VAI isolated and identified micromycetes. The two authors were involved in the writing of the manuscript.

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