Airborne fungi in arctic settlement Tiksi (Russian Arctic, coast of the Laptev Sea)

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Abstract

Biodiversity and number of airborne fungi isolated from indoor and outdoor air of different location in the areas of arctic settlement Tiksi (Russian Arctic) are described. Different locations (coastal areas, landscape, streets of Tiksi, abandoned empty houses, flats, public buildings) were observed. Aeromycota characterized by a significant biodiversity (50 species), but only several species were abundant. Airborne fungal spores concentration (CFU) in Tiksi locations was found low. The maximum spore concentrations were observed in air of the abandoned empty houses (inhabited in the past). Many species common for soil were observed at the samples taken at streets and abandoned buildings. Most of them are also known as inhabitants of building materials. Microfungi CFU at settlement territory was twice as high as natural territory. Phospholipase, albuminase and hemolytic activities of microfungi isolates as well as their relation to temperature were studied. Most of the tested isolates demonstrated high levels of all the tested activities. It was concluded that there is a risk of ‘‘mold’’ allergy diseases for the people especially with weakening of immunity at arctic settlement Tiksi. Main sources of the air contamination in arctic settlements and houses could be many anthropogenic substrates which were colonized by soil fungi.

Key words: airborne microfungi, Arctic, indoor and outdoor air, colony forming units, anthropogenic influence, exoenzyme activity, potential virulence

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Introduction

Fungi are cosmopolitan organisms and colonize various substrates in all climate zones at the Earth. Preliminary contamination and colonization of natural and anthropogenic substrates is connected with the diversity and number of spores or cells of microfungi in the atmosphere (air environment). It was shown that the concentration and diversity of microorganisms in the air are changed with location and altitude (Smith et al. 2009). They vary with time of a day, weather, season, and the presence of local spore sources (Lacey 1981). Microorganisms can spread to various altitudes by different vectors (wind, air traffic, volcanic activity, marine water, anthropogenic ways (Griffin 2004, 2007; Griffin et al. 2011).

Fungal spores are the significant fraction of airborne microbiota. They have important health implications for allergy and asthma sufferers. Mould growth may contribute to sick-building syndrome and other environmental health problems (Singh 2001). Indoor air mycobiota is an important part of bioaerosols in practically all types of buildings and premises. It can affect both biodeterioration of building constructions and human health (Gravesen 1979). During the last decades, a list of saprotrophic fungi reported as new human pathogen has increased significantly (Anaissie et al. 2009).

Extensive studies on spatial and temporal distribution of airborne fungal spores have been conducted in different regions of the world (Marshall 1997a, Ščevková et al. 2010, Grinn-Gofroń et Bosiacka 2015).

It is important to note that a considerable part of the Earth biosphere is constituted by psychrosphere with temperatures under 5ºC (Biedunkiewicz et Ejdys 2011). The active exploration of the Arctic and Antarctic comprising a wide range of human activities has a significant impact on the ecosystems of high-latitude regions. Special attention is paid to the safety of human activity in the severe climate of the inaccessible and remote areas. However, aeromycological studies from the cold World regions are limited and scattered (Johansen et Hafsten 1988; Johansen 1991, Li et Kendrick 1995, Marshall 1997a, b; Bilasiewicz et Czarnecki 1999, Morris et al. 2008, Duncan et al. 2010, Kirtsideli et al. 2011). The goal of this work was to study the airborne fungi biodiversity of natural and anthropogenically altered ecosystems in the local area of the Arctic settlements as well as experimental study of fungal relation to the temperature and their potential pathogenicity. All these make it possible to understand the ways of migration and distribution of microorganisms in the Arctic as well as to estimate the level of anthropogenic influence on polar ecosystems.

Material and Methods

Study area

Tiksi is an urban locality (settlement) and the administrative center of the Bulunsky District of the Sakha Republic (Russia), situated on the Arctic Ocean coast. The name Tiksi means "a moorage place" in Sakha language. Latitude 71° 38’ N, longitude 128° 52’ E. It is one of the principal ports providing an access to the Laptev Sea. Tiksi's winters are very cold with temperatures below zero from October through May. The warmest month of the year is July, with an average temperature of 11.5°C.

January is the coldest month of the year (Fig.1). Tiksi has a very dry climate which can be classified as a desert climate. The majority of Tiksi's precipitation falls during the summer months.

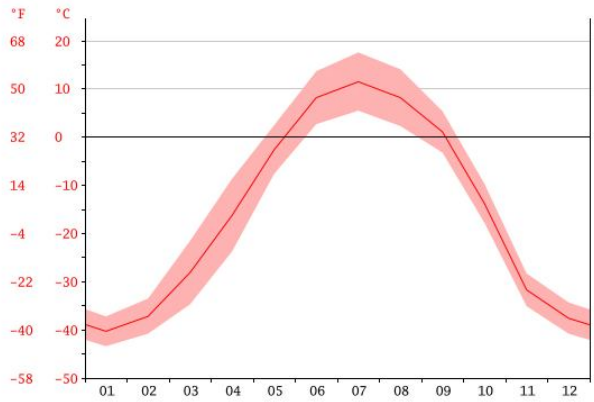

Fig.1. Temperature graph Tiksi ([2]).

Sampling and laboratory study

The investigated area is located in arctic tundra (Fig. 2). Sampling was done in 2012 and 2015 within the seasonal works of the Russian High-Latitude Expedition. More than 350 samples of the indoor and outdoor air were taken by use the sampling devices PU-1B (Russia, HIMKO) and Burkard (United Kingdom, Burkard). Microorganisms from all samples were sedimented on the Petri dishes with agar media (Czapek agar, Saburo agar, meat peptone agar – MPA, ammonia starch agar – ASA). Sampling was made in different locations of the settlement Tiksi (Fig. 2).

Sampling was made in different locations of the settlement Tiksi: 1) coastal areas (control), 2) stone and placard tundra, march (control), 3) central streets of Tiksi, 4) abandoned empty houses (2, 3 and 5 floor buildings), 5) flats, 6) public buildings (shops, post, airport). The sampling was done at the height of approximately 1 m above ground. The volume of air passed through on a Petri dish with the medium was from 100 to 1500 liters, depending on the estimated number of spores in the air. After 10-30 days of cultivation (at temperature 10°, 25°C) colonies were counted. Microfungi carry into pure culture on Czapek agar and identified after sporulation appeared with using optical microscopy approach and standard mycological literature. Stock cultures for further experiments were kept on Czapek agar.

Identification of some species was made by molecular methods by the analysis DNA region containing internal transcribed spacers ITS1 and ITS2. DNA was isolated from 5-day fungal cultures. The DNA region containing the internal transcribed spacers ITS1 and ITS2 was amplified with the primers ITS-1 (5'-TCCGTAGGTGAACCTG CGG-3') and ITS-4 (5'-TCCTCCGCTTAT TGATATGC-3') (Hsiao et al. 2005, Li et al. 2007). The resulting nucleotide sequences were compared by the BLAST program (Basic Local Alignment Search Tool, public domain software,) with the nucleotide sequences available in the open database on the NCBI site.

The names and status of fungal taxa were unified using by database CBS ([1]). Relation of the obtained isolates to temperature factor was studied on Czapek agar and PMA at temperature 3-4°C, 14-15°C, 25°C.

Fig. 2. Location of the study place: 1 – coastal areas, 2 – landscape, 3 – streets of Tiksi, 4 – abandoned empty houses, 5 – flats, 6 – public buildings.

Phospholipase activity (without distinction for phospholipase types) was measured using egg yolk containing medium (Fotedar et Al-Hedaithy 2005). Fungal cultures were incubated at 15°C temperature for 10-30 days. Then the colony diameter and zone of clarification were measured and phospholipase activity coefficient was calculated according to the formula:

$P_z = 1 - D_c(D_c + D_{ca})^{-1}$ *Eqn.*1

P_z – phospholipase activity index, D_c – the colony diameter, D_{ca} – the clarification zone diameter.

Albumenize activity was measured using egg albumen (Bilay 1982).

Hemolytic activity was evaluated by the following method. Human donor blood was defibrinated by shaking with sterile glass beads. Then a 5 ml sample was aseptically added to each 95 ml of blood agar plates at 45°C. Antibiotics (penicillin or erythromycin 0.05 g l^{-1}) were added to prevent bacterial growth. The zone of surrounding the colonies was measured after 10-30 days of incubation and change of medium color (from red to green-brown color) was fixed. The zone of alteration surrounding the colonies was measured after 10-30 days of incubation as zone of clarification (α) or change of color (β) (from red to green-brown color). Hemolytic activity index was calculated by the same formula as the one used for phospholipase activity. All experiments were done in triplicate. Statistical analysis was made by Statistica software.

Results

Biodiversity of airborne microfungi

In the air of the areas of arctic settlement Tiksi, 50 microfungi species were found (*see* Table 1). *Alternaria alternata, Aureobasidium pullulans*, *Chaetomium globosum, Cladosporium cladosporioides, C. herbarum, Geomyces pannorum, Itersonilia pannonica, Rhodosporidiobolus colostri* and four species from genus *Penicillium* were the prevailing species in aeromycota.

Species of microfungi	Coastal areas	Landscape	Streets of Tiksi	Empty houses	Flats	Public buildings
Alternaria spp. *(A. alternata* (Fr.) Keissl., *A. tenuissima* (Kunze) Wiltshire)	6.2	2.3	4.0	5.1	6.2	2.8
Aspergillus spp. *(A. flavus* Link, *A.niger* Tiegh., *A. sydowii* (Bainier et Sartory) Thom et Church)	-	-	2.0	-	6.2	17.8
Aureobasidium pullulans (de Bary) G. Arnaud	15.5	4.6	8.0	1.7	-	-
Chaetomium globosum Kunze	3.1	9.2	8.0	1.7	-	-
Cladosporium spp. (*C. cladosporioides* (Fresen.) G. A. de Vries, *C. herbarum* (Pers.) Link*, C. sphaerospermum* Penz.)	31.0	20.8	24.0	52.7	6.2	5.6
Exophiala jeanselmei (Langeron) McGinnis et A.A. Padhye	9.3	2.3	6.0	3.4	-	2.8
Geomyces pannorum (Link) Sigler et J. W. Carmich. (*Pseudogymnoascus pannorum* (Link) Minnis & D.L. Lindner)	1.5	6.9	4.0	1.7	-	5.6
Penicillium spp. (*P. aurantiogriseum* Dierckx, *P. canescens* Sopp, *P. chrysogenum* Thom, *P. glabrum* (Wehmer) Westling, *P. lanosum* Westling, *P. roqueforti* Thom, *P. simplicissimum* (Oudem.) Thom, *P. spinulosum* Thom)	21.7	29.3	10.0	18.7	18.6	28.0
Rhodosporidiobolus colostri * (T. Castelli) Q.M. Wang, F.Y. Bai, M. Groenew. & Boekhout	-	-	6.0	1.7	9.3	2.8
Total species number	13	21	22	18	17	26

Table 1. Dominant airborne microfungi species and their ability (%) at different location.
* Identification by molecular methods.

The aeromycota of the studied territory was mainly formed by *Ascomycotina* – 82% of species composition (anamorpha, light and dark color – 76%: teleomorpha – 6%). Other groups were represented by *Zygomycotina* – 14% and *Basidiomycotina* – 4%.

In order to compare species composition and population densities at different location of Tiksi settlement, we used a hierarchical clustering tree. Dendrogram shows that the complexes of airborne microfungi are divided into some clusters (Fig. 3). One of them includes control environmental landscape and coastal area near settlement Tiksi. Their proximity may be explained by absent (or insignificant) of anthropogenic influence. The second cluster includes the streets of Tiksi and abandoned empty houses and it was quite close to the control environmental landscape cluster. The next cluster integrates the airborne fungi of living and abandoned empty houses.

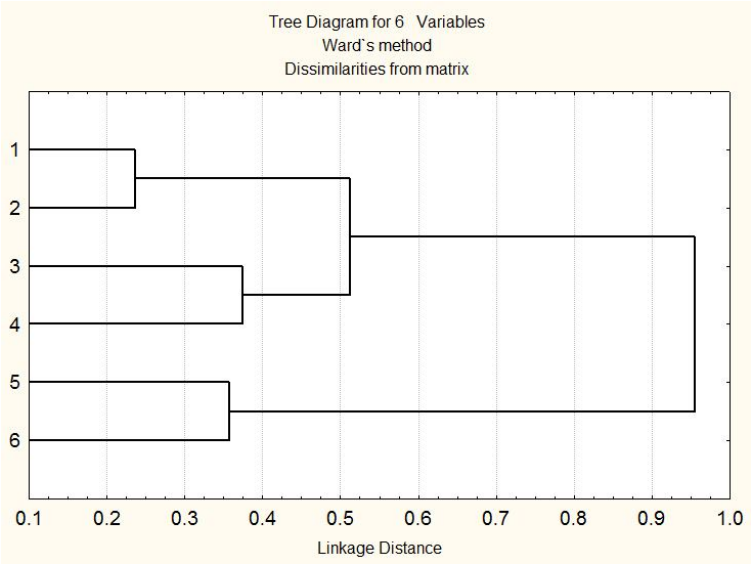

Fig. 3. Cluster analysis of similarities in the distribution of fungal species (aeromycota) in 6 different locations: 1 – coastal areas, 2 – landscape, 3 – streets of Tiksi, 4 – abandoned empty houses, 5 – flats, 6 – public buildings.

The number of viable air fungal propagules (CFU in 1 m^3) varied at different locations (Fig. 4) from 16 (at coast territory) to 445 (at abandoned empty houses). Bacterial colony numbers varied from 42 (at coast territory) to 697 (at public buildings).

Rate of microfungi capable to grow at temperature of 3–4°C reduces from 100% in the air of landscape and coastal area to 87% at indoor air. In contrast, the rate of microfungi capable to develop at 37°C increases from 3% at coastal area to 29% at indoor air. Such microfungi as *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Mucor plumbeus, Mucor racemosus, Penicillium aurantiogriseum, P. chrysogenum, Ulocladium consortiale* were developed at the temperature 37°C.

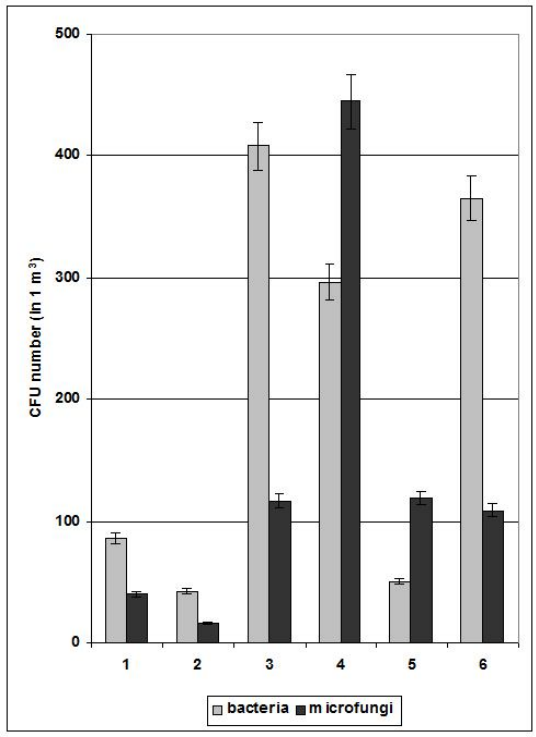

Fig. 4. Number of airborne fungi and bacteria (averages) from the different locations (1 – coastal areas, 2 – landscape, 3 – streets of Tiksi, 4 – abandoned empty houses, 5 – flats, 6 – public buildings).

Enzymatic activity

38 isolates from 18 species of microfungi were tested on exoenzyme activities. The ability to produce exoenzymes in many cases was depended on isolates peculiarities. In some cases, the exoenzyme activity profiles of isolates for the same species were not the same (Table 2).

Phospholipase activity was found in 27 isolates (71%). Among them, 12 isolates showed a high level of enzyme activity varying from 0.17 to 0.70. The other isolates were found positive or weakly positive. The isolates that showed elevated phospholipase activity belong to the following species: *Aspergilus niger, Aspergillus flavus, Geomyces pannorum*, *Mucor hiemalis, Mucor plumbeus, Penicillium aurantiogriseum, P. canescens, P. simplicissimum, Rhodosporidiobolus colostri.* Albuminase activity was found in 2 isolates (5 %).

Hemolytic activity was found in 20 isolates (approx. 53%). Among them 8 isolates exhibited high activity varying from 0.24 to 0.71; and the others were recorded as positive. Isolates that showed high hemolytic activity belong to the following species: *Aureobasidium pullulans, Geomyces pannorum, Penicillium aurantiogriseum, Rhodosporidiobolus colostri*. Only 6 strains (16%) did not show activity for at least one exoenzyme.

Isolates from different location*	Phospholipase activity	Albumenize activity	Hemolytic activity (α)	Hemolytic activity (β)
Alternaria alternata (1)	+	-	-	-
Aphanocladium album (2, 3)	0.21	-	-	-
Aspergilus sp (A.flavus (6*), A.niger* (3*)*	0.57	-	-	+
Aureobasidium pullulans (1, 2, 3, 4)	+	-	0.24-0.71	-
Botryotinia fuckeliana (2)	+	-	-	-
Cladosporium cladosporioides (1, 2, 3)	-\+	-\+	-\+	-
Geomyces pannorum (2, 3)	0.6	-	0.24-0.66	-
Itersonilia pannonicus (3)	+	-	+	-
Mucor spp.: *M. hiemalis* (6) *M. plumbeus* (5)	0.57-0.7	-	-	-
Penicillium aurantiogriseum (3, 4, 6)	0.17-0.59	-\+	0.60	-
P. canescens (1, 4, 5)	0.53-0.59	-	-	-\+
P. simplicissimum (2, 4, 6)	0.63	-	-\+	-
Phaeosphaeria vagans (1, 2)	-	-	-	+
Rhodosporidiobolus colostri (3, 4, 5)	0.43	-	0.67	-
Ulocladium consortiale (4, 6)	-\+	-	-	-

Table 2. Enzymatic activities of airborne microfungi isolates.
Locations: 1 – coastal areas, 2 – landscape, 3 – streets of Tiksi, 4 – abandoned empty houses, 5 – flats, 6 – public buildings.
"+" – presence of enzymatic activity
"-" – absence of enzymatic activity
"-\+" – presence or absence of enzymatic activity for different isolates.

Discussion

Based on these results, we can conclude that the airborne fungal spores concentration in Tiksi locations is generally low. The maximum spore concentrations were observed in air of the abandoned empty houses (left by residents 20-30 years ago). It could be explained by the presence of different cellulose-containing and other anthropogenic materials which are colonized by microfungi in wet periods. Also the absence of ventilation in such places promoted the development and accumulation of microfungi. The high fungal concentration in air at the streets of Tiksi could be connected with several combined factors (large areas with open soil surfaces, strong wind, dust, the presence of vegetation and contamination as result of human activity).

Biodiversity of Aeromycota reached 50 species at the observed territory. However, only several species were found abundant (*Aureobasidium pullulans, Chaetomium globosum, Cladosporium cladosporioides, Exophiala jeanselmei, Geomyces pannorum, Penicillium* spp. and *Phoma glomerata*). *Cladosporium* spores had the highest concentration in the air of coastal area. These data agree with the results obtained

by some researches in different regions such as Antarctica (Duncan et al. 2010) and Canada (Li et Kendrick 1995).

Many species common for soil were observed at the air of streets and empty buildings. Most of them are also known as inhabitants of building materials. The dominant genus in such places was *Cladosporium*. Several medically important fungi, for example *Aspergillus, Botrytis, Candida* and *Penicillium* were identified in the indoor and outdoor air as well. The most common indoor fungal genera identified in this study were *Aspergillus, Alternaria, Penicillium, Mucor, Cladosporium, Rhodosporidiobolus, Itersonilia* and *Ulocladium*. Predominance of *Aspergillus, Penicillium* and *Cladosporium* in indoor air is well supported by several studies in different climates zones in the Earth (Shelton et al. 2002, Curtis et al. 2000, Beaumont et al. 1984, Grinn-Gofron et Mika 2008). It is important to note that microfungi CFU at settlement territory was twice as high as that from a natural territory. Obviously, microorganisms may be transmitted to buildings from outside but the most important sources of microbial contamination are usually within the building. Some species (*Aspergillus flavus, A. sydowii, Rhizopus stolonifer, Sclerotinia* sp., *Ulocladium consortiale*) were identified only in the indoor air. Factors such as building dampness, indoor temperature, surface contamination *etc*. favor the growth and reproduction of microfungi including the pathogenic species (Bornehag et al. 2001). There are clinical evidences that exposure to mold and other dampness-related microbial agents increase the risk of the hypersensitivity pneumonitis, allergic alveolitis, chronic rhinosinusitis and allergic fungal sinusitis ([3]). This is especially important in northern territories where people are constantly under strong cold climate which leads to weakening of immunity.

In this study, we evaluated the expression of phospholipase, albuminase and hemolytic activities using common solid test media. Exoenzymes are often considered among the virulence factors of many human pathogenic fungi (Cutler 1991, Odds 1994, Ibrahim et al. 1995, Ghannoum 2000, Cox et al. 2001, Schaller et al. 2005). The experimental approach based on visual testing of extracellular enzyme activities related to pathogenicity is widely used in medical mycology (Price et al. 1982, Samaranayake et al. 1984, Dagdeviren et al. 2005, Fotedar et Al-Hedaithy 2005, Schaller et al. 2005, Bogomolova et al. 2007). In our work the production of three exoenzymes by the fungi isolated from different locations were examined for assessment of the potential fungal health risk. As the most of the tested isolates demonstrated high levels of all three tested activities, it may be concluded that the risk of ‘‘mold’’ allergy diseases for the people at Tiksi settlemet exists, especially in those exhibiting weakening of immunity. Main sources of the air contamination in arctic settlements and houses could be many different anthropogenic substrates which were colonized by soil fungi.

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