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An analysis of fungal propagules transported to the *Henryk Arctowski* Antarctic Station

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Abstract: During three austral summer seasons, dust and soil from clothes, boots and equipment of members of scientific expeditions and tourists visiting the Polish Antarctic Station *Henryk Arctowski* were collected and analysed for the presence of fungal propagules. Of a total of 60 samples, 554 colonies of fungi belonging to 19 genera were identified. Colonies of the genus *Cladosporium*, *Penicillium* and non-sporulating fungus (*Mycelia sterilia*) dominated in the examined samples. The microbiological assessment of air for the presence of fungi was also conducted at two points in the station building and two others outside the station. A total of 175 fungal colonies belonging to six genera were isolated. Colonies of the genus *Penicillium* were the commonest in the air samples. The potential epidemiological consequences for indigenous species as a result of unintentional transport of fungal propagules to the Antarctic biome are discussed in the light of rapid climate change in some parts of the Antarctic and adaptation of fungi to extreme conditions.

Key words: Antarctic, fungal propagules, unintentional transport.

Introduction

Despite the extreme conditions in the Antarctic, such as low temperature, high salinity, osmotic stress and high doses of UV radiation, microorganisms (including viruses, bacteria and fungi) are the most numerous groups of organisms that colonize diverse habitats (Abyzov 1993; Pearce and Wilson 2003; Onofri *et al*. 2007; Ruisi *et al*. 2007; Finster 2008). Among them, fungi are represented by indigenous species, including endemic species found only in the Antarctic, and show a variety of physiological and morphological adaptations to survive in such extreme conditions (Robinson 2001). With about 1,000 species of fungi reported from the Antarctic and sub-Antarctic region, only 2–3% are considered as endemic species (Bridge *et al*. 2008, 2010). Most of the remaining species are considered to be rather cosmopolitan ones, known from other regions of the world (Vishniac 1993; Selbmann *et al.* 2005; Ruisi *et al*. 2007).

An excellent vector for the transfer of fungal and other propagules to and within Antarctic regions is via humans and associated cargo, including food (Whinam *et al*. 2005; Chwedorzewska 2009; Hughes *et al*. 2011; Lityńska-Zając *et al*. 2012; Chwedorzewska *et al.* 2013). Hughes *et al*. (2011) identified 19 species of fungi on rotting fruit and vegetables, also on the packaging and associated soil, most of which have previously been reported in different regions of the Antarctic. However, approximately 30% of them were not recorded previously from continental Antarctica and the Antarctic Peninsula. In addition, Antarctic regions have recently experienced a growing influx of tourists (Chwedorzewska and Korczak 2010), which also favors the introduction of non-native plant (Olech and Chwedorzewska 2011) and animal species (Chwedorzewska *et al.* 2013), but also microorganisms such as bacteria and fungi, including pathogens that may threaten native organisms (Mercantini *et al*. 1993; Rogers *et al*. 2004; Cowan *et al*. 2011).

The aim of this study was to investigate the extent of the transfer of fungal propagules into King George Island (where the Polish Antarctic Station is located) on clothes, boots and equipment arriving each year in association with scientists and tourists. This is the first project allowing for qualitative and quantitative assessment of transmissible fungal spores to the Antarctic biome.

Materials and methods

The samples were collected during three austral summer seasons: 2007/08, 2008/09 and 2009/10. Members of scientific expeditions and tourists (only one season 2007/08) arriving at the Polish Antarctic Station *Henryk Arctowski* (King George Island, South Shetland Islands, 62°09'S, 58°28'W) were checked for the presence of fungi. In season 2007/08 and 2009/10 samples were taken, respectively, from 20 and 21 people. All outdoor clothing and equipment (bags, backpacks) were thoroughly vacuumed each into a separate standard synthetic vacuum bag. Each sample was tagged, placed in a separate sterile zip lock bag and preserved (by cooling to 4°C) for transportation to Poland for further analysis. In season 2008/09 samples were collected from 19 people. During this season soil, mainly from boots, clothing and other equipment, was collected. Tourists were vacuuming on the ship before going down to land, while the members of the expedition immediately after their descent to land.

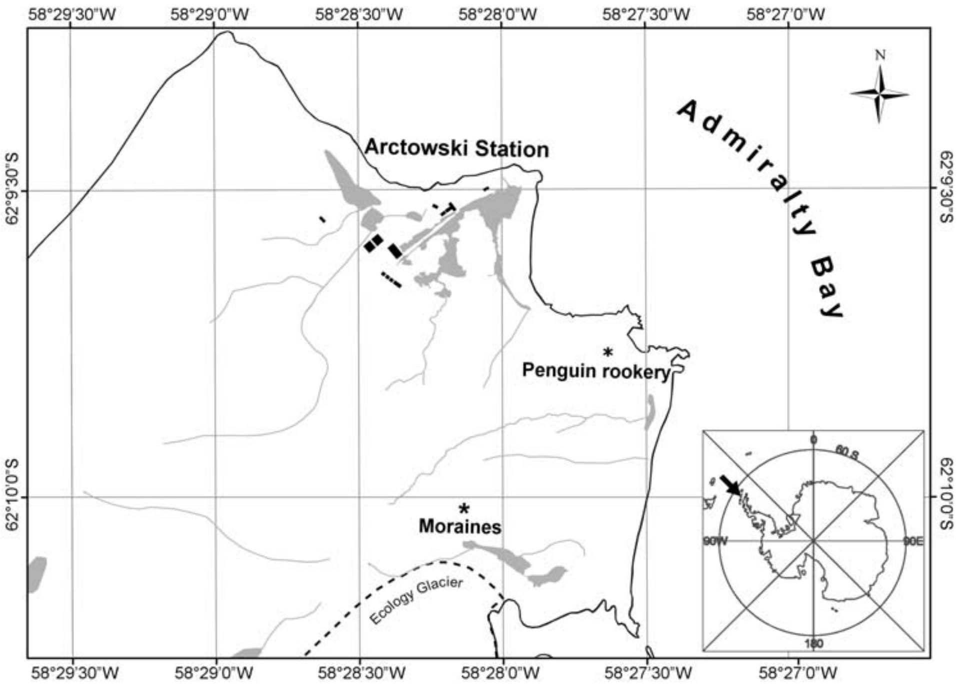

Fig. 1. Map of the study area showing sites used for aerobiological sampling: moraines, penguin rookery (*) and *Arctowski* Station.

Due to a small amount of the dust sample (dust was embedded in the structure of the material), of each vacuum bag three squares were cut out with sterile scissors, each with an area of 1 cm^2. Then the squares were placed separately in test tubes with 10 ml aqueous solution of Triton-X (0.05%), vortexed at maximum speed for 5 min and 0.1 ml plated onto agar medium. Squares cut out from the "clear bags" (bags not used for vacuuming) were treated as a control. Fungi from soil samples were isolated using the standard dilution plate method (Pepper *et al.* 1995). Because different groups of fungi require different conditions for optimal growth (different carbon and nitrogen source, different pH), four isolation media were used for all samples: PDA (Potato Dextrose Agar), MEA (Malt Extract 4% Agar), RBA (Rose Bengal Agar with chloramphenicol) and SDA (Sabourauda Dextrose 4% Agar with chloramphenicol). Each sample was tested using three plates of each of the four media and incubated at 22°C for 5–7 days. Fungal isolates were identified to genus level using morphological taxonomic keys (Gilman 1959; Barnett and Hunter 1972; Domsch *et al*. 1980).

During the 2008/09 season, mycological analysis of the air in the vicinity and inside the station was also conducted. Air from inside of the station was sampled at two points – room in the living quarters and storeroom, and at two points outside the station – in the moraines and penguin rookery (Fig. 1). Airborne fungal spores were determined using the Koch sedimentation method in accordance with a Polish Standard (PN-89Z-04111/03). Air microorganisms were settled gravitationally, directly on open Petri dishes with Sabouraud dextrose agar (Envirocheck Settle Plate, Merck). At each point 20 plates were used. Then the plates were incubated at 25°C for 5 to 7 days. Concentration of fungal spores in 1 m^3 of the air determined as colony forming units (CFU) was calculated using Omeliański formula modified by Gogoberidze (PN-89Z-04111/03).

$$CFU/m^3 = \overline{a} \times 10^4/p \times t \times 0.2$$

$\overline{a}$ – average number of fungal colonies on the Petri plate
p – the surface of the Petri plate (in cm)
t – the time of the Petri plate exposure (in minutes)
0.2 – exposure time conversion factor of Petri plates

Results

During the 2007/08 season, 20 samples taken from people's clothing and equipment were analyzed and in total 189 fungal colonies (isolates) belonging to 11 genera were found, in the 2008/09 (19 samples) 252 colonies were represented by 14 genera, and in the 2009/10 season (21 samples) 113 colonies belonging to eight genera were found (Table 1).

Fungi were isolated from almost all the samples. No fungi were isolated only from one sample collected during the 2008/09 season and one other collected during the 2009/10 season, which represents approximately 5% of all analyzed samples. Colonies of the genus *Cladosporium* (100 isolates) were the commonest in the examined samples in the 2007/08 season, while, during the 2008/09 and 2009/10 seasons, colonies of *Penicillium* were the most commonly isolated (103 and 46 isolates respectively*). Alternaria*, *Geotrichum*, *Aspergillus* and non-sporulating fungi (*Mycelia sterilia*) were also abundant in the samples. Fungi of other genera were found occasionally (Table 1).

The most frequently isolated fungus for each of the seasons was also observed in the largest number of samples. The only exception was the genus *Alternaria* in the 2007/08 season, where 35 colonies came from only two samples (representing 10% of all analyzed samples in this season). In general, the most commonly isolated fungi were detected in more than 50% of the samples (Table 2).

A total of 175 fungal colonies belonging to six genera were isolated from the air samples at the four sampling sites (Fig. 1, Table 3). Yeast-like fungi and non-sporulating fungi (*M. sterilia*) were also identified. Both in the room and the storeroom *Penicillium* was the most represented genus, with 16 and 78 colonies respectively. In the storeroom colonies of yeast-like fungi were also abundant (47 colonies). At the moraines sampling site (Table 3) only one colony of the genus

Table 1

The number of isolates of different fungal genera from four different media in three seasons.

genus	season		
	2007/2008	2008/2009	2009/2010
Acremonium		1	
Alternaria	35		15
Aspergillus	5	11	12
Botritis	2	3	
Cladosporium	100	18	1
Chaetomium	8		
Chloridium	6	3	
Fusarium		2	
Geotrichum	1	52	1
Hyalodendron		1	
Monocillium	1	1	
Mucor	4		13
Penicillium	11	103	46
Torula		3	
Trichocladium			5
Trichoderma	1	1	
Scopulariopsis		5	
Verticillium		2	
Mycelia sterilia	15	46	20
Total	189	252	113

Table 2

The frequency of isolation of most often detected fungi.

season	most frequently isolated fungi	no of samples (no of positive samples*)	no of isolates	frequency of isolation (%)**
2007/2008	*Cladosporium*	20 (15)	100	75
	Alternaria	20 (2)	35	10
	Mycelia sterilia	20 (5)	15	25
	Penicillium	20 (6)	11	30
2008/2009	*Penicillium*	19 (15)	103	79
	Geotrichum	19 (12)	52	63
	Mycelia sterilia	19 (14)	46	74
	Cladosporium	19 (11)	18	58
	Aspergillus	19 (8)	11	42
2009/2010	*Penicillium*	21 (14)	46	67
	Mycelia sterilia	21 (9)	20	43
	Alternaria	21 (3)	15	14
	Mucor	21 (2)	13	10
	Aspergillus	21 (10)	12	48

* – number of samples from which the fungus was isolated

** – the ratio of the number of positive samples to the total number of samples

Table 3

The number of fungal colonies isolated from the air inside and outside the station.

genus	sampling site			
	room	storeroom	moraines	penguin rookery
Alternaria	1			
Aspergillus	1			
Bispora		1		
Cladosporium	1			
Penicillium	16	78	1	
Verticillium		1		
Mycelia sterilia	15	2	1	
Yeast-like fungi	10	47		
Total CFU/m^3	44 1.7×10^2	129 5.1×10^2	2 0.8×10^1	0 0

Penicillium and one belonging to *M. sterilia* was isolated. At the penguin rookery sampling site no fungal colonies were isolated.

Discussion

This study shows that fungal spores can be transported to the Antarctic biome on people's clothing and expedition equipment in a rapid manner. The most common fungal genera were *Penicillium*, *Cladosporium*, *Alternaria* and *Geotrichum*. These are cosmopolitan and ubiquitous fungi, widespread in nature, occurring in many regions of the world, including alpine and polar areas (Bridge *et al*. 2010). They grow well on many substrates including decaying organic material, plant debris, soil and food stuffs, but also occur in the air as part of the bio-aerosol (Domsch *et al*. 1980; Marshall 1997). Therefore, the question is whether fungi introduced unintentionally to Antarctic regions, on personnel clothing and equipment, should be considered as alien species? It is difficult to answer this question. Numerous mycological studies conducted in different regions of the Antarctic show that fungi of the genus *Cladosporium*, *Penicilium*, *Aspergillus* are found relatively frequently in soil and air, and are even isolated from within deep layers of polar ice that were originally deposited several thousands years ago (Del Frate and Caretta 1990; Baublis *et al*. 1991; Marshall 1997; Azmi and Seppelt 1998; Tosi *et al*. 2002; Arenz *et al*. 2006; D'Elia *et al*. 2009; Rosa *et al*. 2009; Zucconi *et al*. 2012). However, different strains of the same fungal species (strains from both within and outside the Antarctic) may have different physiological characteristics and unknown consequences on the Antarctic indigenous species and ecosystems (Litchman 2010; Cowan *et al*. 2011). Nevertheless, the fungi which are accidentally introduced to colonize a new environment, besides viable propagules, must, above all, have the ecophysiological characteristics required for survival in the polar environment and to allow the species to grow and establish (Ellis-Evans and Walton 1990; Pearce *et al*. 2009).

During the austral summer the mean air temperature in the vicinity of *Arctowski* Station may reach 2.5°C (maximum 10.4°C, minimum -1.3°C) with wide daily fluctuations including temperatures dropping below freezing (Kejna 2008; Kejna *et al.* 2013). Recently, the Antarctic Peninsula is one of the fastest warming regions on the Earth. The largest annual warming trends were found in the western and northern parts of the Antarctic Peninsula, with temperatures at *Faraday/Vernadsky* Station increasing at a rate of +0.56°C over the decade in the period 1951–2000 (Turner *et al.* 2005). During sunny days the soil surface can heat up to temperatures of 10–15°C or even higher (Davey *et al.* 1992), and thus create optimal conditions for the development of some non-native (considered as psychrophilic) species of the genus *Penicillium* or *Cladosporium*. However, most of the unintentionally introduced fungi are either psychrotolerant or psychrotrophs, and even mesophilic species. Most filamentous fungi have a short reproductive cycle that can last only a few days and their life cycle can be completed by the production of a vast number of spores that can spread, but it is rather unlikely for them to significantly contribute to any aspect of nutrient cycling and to have any impact on the microbiological community structure or function, owing to their low metabolic activities (Cowan *et al*. 2011).

In the examined samples non-sporulating colonies *M. sterilia* were found relatively often. During the 2008/09 season, 46 sterile colonies were isolated from 14 of the 19 soil samples (74% of the total). It is supposed that the formation of sterile mycelia is one of the mechanisms of fungal adaptation to low temperatures or to a lack of nutrients (Robinson 2001). Other adaptation of fungi to extreme conditions may also shorten the life cycle or, conversely, lengthen it. For example, *Penicillium hirsutum* (growing on garlic in storage) showed both delayed germination of spores and sporulation at lower temperatures (from 4 to -2°C) than in higher ones (10 and 20°C), whereas at a temperature of -4°C it showed a total loss of sporulation (Bertolini and Tian 1996). Reduction of metabolism could be the first step of adaptation to extreme condition, while physiological mechanisms conferring cold tolerance in fungi are more complex (Robinson 2001). Fungi *M. sterilia* are often isolated from various habitats and regions, within both the Arctic (Hyvärinen *et al*. 2001; Salonen *et al*. 2007) and the Antarctic. Azmi and Seppelt (1998) in the Windmill Islands region found that the non-sporulating fungi *M. sterilia* were the most frequently isolated fungi from samples of soil, mosses, algae and lichens. Similarly, in earlier studies conducted by Fletcher *et al*. (1985) in Mac. Robertson and Enderby Lands (East Antarctica), the sterile mycelial fungi comprised respectively 47 and 60% of isolates.

The austral summer, from December to March, is a period of increased influx of tourism and research groups in some parts of the Antarctic. Human activities are focused mainly on ice-free areas of the maritime Antarctic, where there are around half of the research stations, and where climatic conditions are not as severe as in the continental regions. Thus, the fungal spores unintentionally introduced into the polar stations may find appropriate conditions for development. The high density of fungal spores inside the station, where there are good conditions for their development (*i.e.* on food waste), shows that these places can be a good source for them to spread to the nearby surroundings. Mycological analysis of the air was carried out in the vicinity and inside the *Arctowski* Station previously in 1985 and 1999 (Czarnecki and Białasiewicz 1987; Białasiewicz and Czarnecki 1999). These studies showed that fungi of the genus *Penicillium* were the most frequently observed inside the station, which supports other research performed at *Syowa* Station (Nakashima *et al*. 2003). In our study we received a total of 94 isolates of *Penicillium* genus, of which 78 were isolated from the fruits and vegetables storeroom. As Białasiewicz and Czarnecki (1999) suggested, the source of fungi collected inside the station could be brought by human vectors, as well as originate from the natural environment outside the station. However, other studies have also indicated that research stations and human activity in the vicinity of research stations may be a source of microorganisms released into the local Antarctic environment (Hughes *et al*. 2010; Pearce *et al*. 2010).

The fact of unintentional transport of fungal propagules along with people arriving at the polar stations, regardless of whether these fungal species are considered to be alien or not, is a dangerous phenomenon since many of the species recorded in the studied samples are potentially pathogenic to plants (Hughes *et al*. 2011) and warm-blooded animals (Wicklow 1968; Mercantini *et al*. 1989). For many indigenous populations of the Antarctic flora and fauna, geographical isolation is the reason why fungi associated with human activities in the Antarctic can be novel pathogens. Thus, the flora and fauna of the polar regions may be particularly sensitive to infections, which could have devastating consequences on the indigenous biota (Rogers *et al.* 2004; Weimerskirch 2004; Barbosa and Palacios 2009).

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