Fungal species associated with fruit and vegetables transported to the J.G. Mendel station and the influence of UV-C treatment on their fungal community

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Abstract

The aim of this study was to investigate the fungal community associated with fruits and vegetables transported into the Antarctic region and observe qualitative changes of their surface mycobiota after UV-C treatment. This measure is used to prevent the post-harvest diseases of stored fruits and vegetables and reduce the risk of introducing non-native species to the Antarctic environment. In total, 82 strains of filamentous fungi were isolated from the surfaces of 64 pieces of fresh fruits and vegetables before and after their UV-C treatment. They were assigned to the genera *Penicillium*, *Fusarium*, *Mucor*, *Cladosporium*, and *Acremonium*. After the UV-C treatment of the examined fruits and vegetables, spores of the genera *Fusarium*, *Cladosporium* and *Acremonium* were not detected, while spores of the genera *Penicillium* and *Mucor* were more resistant and stayed viable after the treatment. *Penicillium* strains prevailed in the examined samples. Their introduction to the Antarctic environment could represent a potential risk for endemic autochthonous organisms.

Key words: non-native, fungi, fresh food, Antarctic region, UV-C treatment, post-harvest diseases

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Introduction

The Antarctic continent is frequently described as the last unspoiled region of Earth. However, an increase in human activities such as tourism and scientific programs over the past three decades has affected the Antarctic environment. Several thousand researchers and tourists visit the continent each year (Onofri et al. 2007). Human impacts have reflected by changes in Antarctic microbial communities including viruses, bacteria, archaea, algae, fungi and microeukaryotes. The microbial settlement is strongly affected by the geographic isolation of the Antarctic continent, extreme climatic conditions and the availability of nutrients in the environment (Ruisi et al. 2007).

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Most Antarctic filamentous fungi represent common mesophilic, anamorphic species widespread in the biosphere (Onofri et al. 2007). Some of them are transported to Antarctica by natural means such as wind, ocean debris, birds and marine mammals (Hughes and Convey 2012) but they are unable to grow under Antarctic conditions, while others, termed indigenous, are well adapted and able to grow and reproduce even at low temperatures (Ruisi et al. 2007). More than 1000 fungal species were reported from the Antarctic and sub-Antarctic regions but only 2-3% of them are considered to be endemic (Hughes et al. 2018). Increasing human activities are another mean for the transportation of allochthonous biological material to Antarctica. Microorganisms are introduced in association with cargo, fresh food, personal clothings, building materials, vehicles, aircraft and ship holds (Hughes and Convey 2012, Hughes et al. 2018). The dissemination of non-native species in Antarctica is recently the most discussed issue associated with human activity ([1] - COMNAP and SCAR 2010, Cowan et al. 2011, Hughes et al. 2011, 2015, 2018; Osyczka et al. 2012, Augustyniuk-Kram et al. 2013, [2] - CEP 2017, Galera et al. 2018). Human pollution is particularly noticeable near the scientific stations and locations visited by tourists. So far, the investigation of the ecological impacts of humans on the Antarctic ecosystems has focused on the importation of plant propagules and invertebrates (Hughes et al. 2010, Lityńska-Zając et al. 2012, Chwedorzewska et al. 2013, Huiskes et al. 2014, Molina-Montenegro et al. 2015) but less attention has been paid to the introduction of fungal propagules. To the best of our knowledge, only the few following studies dealing with this issue were published. Czarnecki and Bialasiewicz (1987), Osyczka et al. (2012) and Augustyniuk-Kram et al. (2013) analysed of fungal propagules from the air, food, timber, clothes, boots and equipment transported to the H. Arctowski Polar Station. Hughes et al. (2011, 2018) focused on fungi associated with fresh produce (fruit and vegetables) and wooden cargo packaging transferred to the Antarctic region.

Fruit and vegetables are mainly colonised by fungi causing post-harvest diseases. Many of these fungi are present at anamorphic stage and belong to the phylum Ascomycota. Important post-harvest pathogens are represented by the anamorphic genera *Penicillium*, *Aspergillus*, *Botrytis*, *Fusarium*, *Alternaria*, *etc*. (Coates et al. 1997).

To protect the Antarctic biodiversity against the unintended introduction of not-native species to that region, the Committee for Environmental Protection compiled a Non-native species manual ([2] - CEP 2017). This manual includes practical guidelines and resources to support the prevention of the introduction of non-native species. Hughes et al. (2011) suggested a provisional list of measures reducing the risk of introductions of non-native species to the Antarctic region associated with fresh food. One of the measures regarding the storage of fresh fruits and vegetables on a station is the use of germicidal lamps (UV-C) in storage areas.

UV-C radiation is lethal to most microorganisms, including bacteria, viruses, fungi, yeast and algae (Begum et al. 2009). In practice, it is used for a treatment of air, liquids and surfaces of objects where a sterile environment is needed. In many studies, the germicidal effect of the UV-C radiation on fungi causing a post-harvest *decay* has been demonstrated (Rodov et al. 1992, Valero et al. 2007, Gündüz and Pazir 2012, Uyar and Uyar 2018).

The aim of this work was to investigate fungal communities associated with fruits and vegetables transported to the Johann Gregor Mendel Station located on James Ross Island and observe changes in these communities after the UV-C treatment.

Material and Methods

Sample collection

In total, 8 kinds of fresh fruits and vegetables were tested for the presence of filamentous fungi within the polar expedition during austral summer season 2012/13. All fruits and vegetables were purchased in Punta Arenas (Chile) shops and transported by ship to the J. G. Mendel station (60° 48′ 2.3″ S; 57° 52′ 56.7″ W) located on James Ross Island. Examined fruits and vegetables included lemons (*Citrus limon*), grapefruits (*Citrus paradisi*), limes (*Citrus limetta*), oranges (*Citrus limonia*), apples (*Malus domestica*), potatoes (*Solanum tuberosum*), garlic (*Allium sativum*) and cabbage (*Brassica oleracea* convar. *capitata* var. *alba*). Immediately after unpacking, 8 pieces of each kind of fresh food were randomly wiped by sterile cotton swabs Fungi-Quick into the transport medium for mold and yeast. Afterwards, all wiped pieces of the fruits and vegetables were treated by a germicidal lamp from two sides. The treatment time was 5 min. from each side (Gündüz and Pazir 2012). Subsequently, all pieces of the fruits and vegetables were again randomly wiped by the transport swabs. The swabs were stored in the refrigerator at 5°C and then transported to the laboratories of the Czech Collection of Microorganisms (CCM - Masaryk University, Brno, [3]).

Isolation and identification

In total, 128 swab specimens were qualitatively analysed in the CCM laboratories. The swab specimens were inoculated onto the surface of three isolation media: PDA (Potato Dextrose Agar), DRBC (Dichloran Rose Bengal Agar with chloramphenicol) and MEA (2% Malt Extract Agar). Each sample was seed on two plates of each of the three media and incubated at 25°C for 5–10 days. Morphologically different colonies from each sample were subcultured on MEA in order to obtain pure cultures. The identification of fungal samples was done on the basis of traditional methods of macroscopic and microscopic examination. Fungal isolates were identified using morphological taxonomic keys (Zycha et al. 1969, Domsch et al. 1980, Frisvad and Samson 2004, Leslie and Summerell 2006). *Penicillium* and *Fusarium* species identification was supported by biochemical analyses using the Biolog FF MicroPlate™ (Biolog, USA) as a complement to the traditional methods. Publications by Bridge et al. (2010), Onofri et al. (2007) and studies dealing with Antarctic mycobiota published after 2010 were used to determine if the identified fungal species had previously been recorded from Antarctica.

Results

In total, 82 isolates of filamentous fungi were retrieved from the surface of the fresh fruits and vegetables. Fifty-four colonies were isolated before and 28 colonies after the UV-C treatment. Identified fungi were represented by 5 genera. The majority of the isolates belonged to the genus *Penicillium* (72%) and the remaining isolates belonged to the genus *Fusarium* (16%), *Mucor* (7%), *Cladosporium* (4%) and *Acremonium* (1%). Among them 45 strains were assigned into 13 species within the genera *Fusarium*, *Mucor,* and *Penicillium.* Roughly two thirds of them have previously been recorded from the Antarctic regions (Table 1). *Penicillium expansum* and *Penicillium solitum* were the most frequently isolated species and also occurred across the largest number of fruits and vegetables kinds (Table 1). *Mucor* species were observed only from potatoes before but also after the UV-C radiation. Isolates belonging to the genus *Fusarium* were identified to 3 species. Lemons were the largest source of *Fusarium* species including *Fusarium solani* and *Fusarium proliferatum*. *Fusarium* and remaining ascomycetes were not isolated from any samples after the exposure to the UV-C radiation. By contrast, *Penicillium* species dominated after the UV-C treatment (Fig. 1). No fungal species was isolated only from garlic after the treatment. The UV-C radiation led to the isolation of some species that had not been recorded prior to the treatment due to dominance of competing fungal species. All identified fungi isolated before and after the UV treatments are listed in Table 1.

Fungal taxa	**lemon**	**grape-fruit**	**lime**	**orange**	**apple**	**pota-toes**	**garlic**	**cab-bage**
Acremonium sp.					B			
Cladosporium sp.		B		B		B		
*Fusarium oxysporum**						B	B	
*Fusarium proliferatum**	B							B
Fusarium solani	B							
Fusarium sp.	B							
*Mucor hiemalis**						B/A		
Mucor sp.						A		
Penicillium aethiopicum				A				
Penicillium allii							B	
*Penicillium chrysogenum**	A							A
*Penicillium brevicompactum**						B		
*Penicillium expansum**	A		B		B/A			A
*Penicillium italicum**			B/A		A			
*Penicillium jensenii**			A					
*Penicillium polonicum**			B				B	
*Penicillium solitum**		A	B	A	B/A	B		A
Penicillium spp.	A	A	B	B/A	B/A	B		

Table 1. Fungal taxa isolated from the fruits and vegetables before and after the UV-C treatment, species previously recorded from Antarctica are marked with an asterisk*; B - species isolated before UV-C treatment; A - species isolated after UV-C treatment.

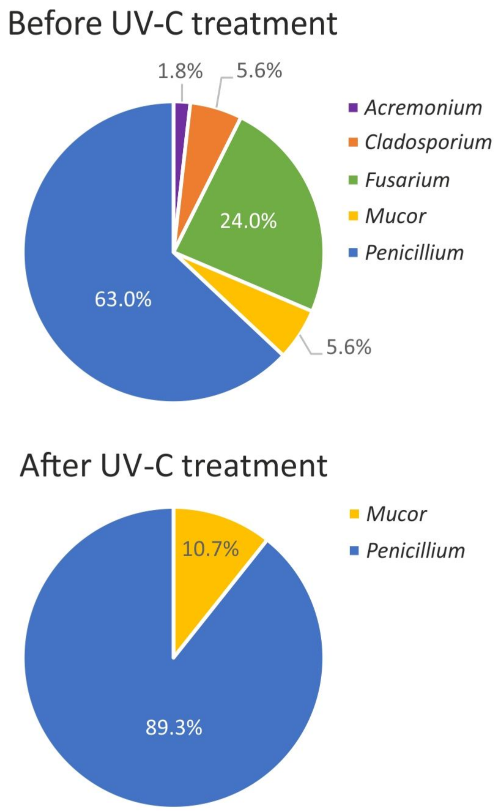

Fig. 1. Incidence of the fungal genera on the fruits and vegetables before and after the UV-C treatments.

Discussion

This mycological study implies that the importation of fresh food within polar expeditions to the Antarctic region contributes to the spread and introduction of fungal species allochthonous for the Antarctic environment. Less than one-third of the identified fungi isolated from the fruits and vegetables were not recorded previously from Antarctica. Similar results were obtained also by Hughes et al. (2011) who examined the microbial decomposition of fresh produce transported to Antarctica and found out that 28% of the food items showed microbial infection and 30% of the identified fungi were not recorded previously from the Antarctic region. Most of the fungal taxa identified in our study are commonly found on fresh food or in soil with cosmopolitan distribution outside Antarctica. Some of the identified species are known to be phytopathogenic species causing plant diseases (*Fusarium*) and spoilage of stored food (*Penicillium*). *Penicillium italicum* (blue mould), the most frequently occurring species on the examined limes, is the main pathogen of *Citrus* species causing the post-harvest rotting of around the world (Pitt and Hocking 1997). In recent years, it has caused significant economic losses in Mexican lime production (Hernández-Montiel et al. 2010). *Penicillium italicum* has been recorded from Antarctica by Kostadinova et al. (2009) who isolated this species from soil on Livingston Island (part of the South Shetlands archipelago). *Penicillium expansum* is another species that causes spoilage of fresh fruits and vegetables in storages. This species was isolated across the broad spectrum of the fruits and vegetables (lemons, limes, apples and cabbage) transported to the J. G. Mendel station. It has been predominantly reported from rotting apples and pears (Pitt and Hocking 1997) but its isolation from fresh vegetables is rare. For example, Lugauskas et al. (2005) reported occurrence of *P. expansum* on carrots, onions and cabbages obtained from storehouses. *Penicillium expansum* is able to grow well at low temperatures therefore it is widespread in all Antarctic regions (Hughes et al. 2011). *Penicillium solitum* representing a mesophilic and psychrotolerant species was another frequently isolated species from many samples examined in this study (grapefruits, oranges, apples, potatoes and cabbage). This species is known as a significant pathogen of pomaceous fruits causing apple spoilage (Pitt and Hocking 1997) but it has been reported also from marine sediments (Gonçalves et al. 2013) and moss turf (Bridge et al. 2010) sampled in the Antarctic Peninsula and continental Antarctica. Both aforementioned *Penicillium* species were also retrieved from fresh food transported to Antarctica (Hughes et al. 2011). *Penicillium chrysogenum* isolated from the lemons and cabbage in our study is a ubiquitous species often occurring in Antarctic environmental samples but it was reported from fruit stored in the Polish H. Arctowski Polar Station as well (Czarnecki and Bialasiewicz 1987). Hill et al. (1995) indicated this species as a major cause of spoilage of foods transported in shipping containers. *Penicillium brevicompactum* is another ubiquitous *Penicillium* species widespread in the Antarctic environment. This species was obtained from the examined potatoes in our study. Both, *P. chrysogenum* and *P. brevicompactum* were also presented in soil samples collected on James Ross Island (Laichmanová et al. 2009). *Penicillium allii* and *Penicillium polonicum* were isolated only from garlic transported to the J. G. Mendel station. *Penicillium allii* has been reported as the cause of a destructive rot of garlic in Argentina (Valdez et al. 2009). This species was not reported from Antarctica so far, while *P. polonicum* was isolated by Alborés et al. (2018) from the soil and moss sampled on the Fildes Peninsula, King George Island. *Penicillium aethiopicum* isolated from the oranges in our study is known only from Southeast Asia commodities (Pitt and Hocking 1997). *Penicillium jensenii* isolated from the limes in this study is widely distributed mainly in temperate regions (Domsch et al. 1980) however it was also recorded from continental Antarctica (Onofri et al. 2007). The mycological analyses of the dust and soil from clothes, boots and equipment of scientists and tourists visiting the Polish H. Arctowski Polar Station showed the domination of the genus *Penicillium* in the examined samples as well (Augustyniuk-Kram et al. 2013). Based on the observations from multiple studies, penicillia were suggested as biological indicators of human contamination of Antarctica (Onofri et al. 2007). Also our data showed that strains of the genus *Penicillium* dominated over the remaining identified genera isolated from the fruits and vegetables transported to the J.G. Mendel station. *Penicillium* spp. also dominated in soil samples collected on James Ross Island within three polar expeditions in 2007–2009 (Laichmanová et al. 2009).

Most research stations and visiting tourist areas are situated on the coast. Factors which increase the risk of introduction of allochthonous species to the Antarctic region are favourable coastal climate, fast climate changes in some Antarctic regions, morphological and physiological characteristics of introduced fungi, including their ability to germinate and grow at low temperatures, short reproductive cycle and production of huge number of easily spreading spores (Hughes et al. 2011, Augustyniuk-Kram et al. 2013).

The J. G. Mendel station carries out measures to reduce this risk by using UV-C treatment of fruits and vegetables transported to the station. Simultaneously, the UV-C treatment prevents fruits and vegetables decay during storage and extends the shelf life of stored food. In this study, the UV-C irradiation effectively inactivated spores of the genera *Fusarium*, *Cladosporium* and *Acremonium* which have not been isolated after the treatment from any samples, while spores of the genera *Penicillium* and *Mucor* seems to be more resistant. These changes of mycobiota induced by the UV-C treatment led to the isolation of certain species that had not been recorded prior to the treatment (for example *Penicillium* spp. on lemons). The majority of the *Fusarium* species isolated from lemons in our study before the treatment are known as pathogens on citrus presented in almost all citrus orchards all over the word (Adesemoye et al. 2011). The occurrence of *Fusarium* species in Antarctica is not well-known because most of the isolated species remain unidentified. *Fusarium proliferatum,* the most frequently isolated species from lemons, is known as endophytic fungus associated with *Colobanthus quitensis* (Rosa et al. 2010) and *Fusarium oxysporum* obtained from tested potatoes was reported from wood (Arenz et al. 2006) and soil (Onofri et al. 2007) in continental Antarctica.

Conclusion

The results obtained in the frame of the present study showed that fruits and vegetables might be an important source of non-native fungal species which could spread into the Antarctic environment and cause changes in the microbial communities. UV-C radiation is a gentle treatment non-polluting the environment by residual compounds. Our results showed that it prevents the germination and growth of fungi on the surfaces of fruits and vegetables and thus the introduction of non-native species to the Antarctic environment.

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