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ORIGINAL PAPER

Antarctic rocks from continental Antarctica as source of potential human opportunistic fungi

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Abstract We assessed the diversity of culturable fungi associated with rocks of continental Antarctica to evaluate their physiological opportunistic virulence potential in vitro. The seventy fungal isolates obtained were identified as nine species of *Acremonium*, *Byssochlamys*, *Cladosporium*, *Debaryomyces*, *Penicillium,* and *Rhodotorula*. *Acremonium* sp., *D. hansenii*, *P. chrysogenum*, *P. citrinum*, *P. tardochrysogenum,* and *R. mucilaginosa* were able to grow at 37 °C; in addition, *B. spectabilis* displayed a high level of growth at 37 and 45 °C. Thirty-one isolates of *P. chrysogenum*, *P. citrinum,* and *P. tardochrysogenum* were able to produce partial haemolysis on blood agar at 37 °C. *Acremonium* sp., *P. citrinum,* and *P. tardochrysogenum* showed spore sizes ranging from 2.81 to 5.13 µm diameters at 37 °C. Of these, *P. chrysogenum* and *P. tardochrysogenum* displayed macro- and micro morphological polymorphism. Our results suggest that rocks of the ultra-extreme cold and dry environment of Antarctica harbour cryptic fungi phylogenetically close to opportunistic pathogenic and mycotoxigenic taxa with physiologic virulence characteristics in vitro.

Keywords Antarctica · Extremophile · Taxonomy · Virulence factors

Introduction

Antarctica has some of the most diverse and pristine environments on the planet, which include habitats that combine cold, dry, oligotrophic conditions with radiation extremes (Gonçalves et al. 2012). These unusual environments offer new opportunities to discover extremophile microorganisms and study their abilities to survive at the limits of life (Santiago et al. 2012). Among the biota of Antarctica, these microorganisms, represented by bacteria, viruses, protozoa and fungi, occur in different substrates and seem to have important roles in the ecological processes of the region (Ruisi et al. 2007).

Fungi are highly adapted to their habitat and are able to survive in harsh environments with extreme temperatures, prolonged desiccation, and high solar irradiation. They adapt by changing their physiology and morphology through the production of enzymes, melanin, and micosporins (Gostincar et al. 2012). Among the fungi groups, those associated with rocks have been studied due to their capacity to survive on rock surfaces, which represent a unique habitat where rapid changes in radiation, temperature, water, and nutrients occur, representing a challenge to microbial survival in different environments throughout the world (Gueidan et al. 2008).

Few fungal species have been described as causative agents of mycoses; however, unknown fungi present in unexplored regions may display pathogenic virulence

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factors. For this reason, they may threaten plants, animals, and humans. Continental Antarctica represents an extreme environment of the planet with limited outside influences, where the primary production in most open soil areas is largely restricted to cryptoendoliths and hypoliths (Arenz and Blanchette 2011). Antarctica represents a pristine and isolated region of the world, where, in the last several years, several studies have reported the presence of different microbial communities living under extreme conditions. However, there is currently a little knowledge regarding the virulence factors of these microorganisms. For this reason, the aims of our study were to recover and characterize the fungal community living within the rocks of continental Antarctica and evaluate their virulence potential to humans.

Methods

Study area

The rock samples were collected in the Heritage Range that forms the southern part of the Ellsworth Mountain system (Suppl. Fig. 1), consisting of scattered ridges and peaks of moderate height, escarpments, hills, and nunataks. These are the various units of relief set off by numerous intervening glaciers, such as the Union Glacier. The climate in the Heritage Range is typical of the low plateau of continental Antarctica; temperatures above zero persist only for a few hours per day and for less than 30 days during the Summer; in addition, a little precipitation and strong katabatic winds are common (King and Turner 1997). Twenty-seven rock samples from nine different places were collected in the Summer of 2013 from ice-free areas of the Union Glacier region in the southern Heritage Range. Samples were collected using sterile gloves, placed in Whirl–Pak bags (Nasco, Ft. Atkinson, WI, USA) and kept at −20 °C in a sterilized box until transportation to the laboratory at the Federal University of Minas Gerais, Brazil. There, the samples were processed to isolate the fungi.

Rock characterization

Petrographic analyses were completed to identify the types of rocks and their main features. The description of the collected samples was made at the macro and microscopic scale, using “hand samples” and “thin sections”, respectively. The mineral constituents, colours, textures, and structures were analyzed. In the thin sections, optical microscopic investigations were carried out using a Zeiss trinocular optical microscope (Axiophot model) with an integrated digital camera. The most representative sites were selected for microstructural and microchemical studies. These studies allow to obtain the chemical composition of selected areas and points in thin sections using an energy dispersive system (EDS) coupled in a scanning electron microscope (SEM—QUANTA FEI 3D). The thin sections were coated with carbon film. The operating conditions were an acceleration potential of 15 kV and current of 20 nA. The following elements were determined in the microchemical analysis: Na, K, Mg, Ca, Fe, Al, and Si. Microchemical maps were produced in which the coloured portions in the images indicate the presence of specific chemical element.

Fungal isolation

The rocks were washed with Extran detergent (Merck, USA) and disinfected using alcohol 70%, and the inner fragments of 1–2 cm of each rock powdered using a blender for at least 10 min (Gonçalves et al. 2015). After that, 1 g of each sample was added to 9 mL of 0.85% NaCl. Then, 100 µL of a 10^{-1} dilution was inoculated onto YM (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 2% glucose, 2% agar), MYEA (2% malt extract, 0.2% yeast extract, 2% agar), DG18 (Oxoid, USA), and DRBC (Oxoid, USA) media containing 100 µg mL^{-1} chloramphenicol (Sigma). The mixture was incubated at 15 °C for 60 days. Fungal colony-forming units (CFUs) were counted, and subcultures were made of all of the morphologically distinct colonies from each sample. The subcultures were grouped into different morphotypes according to their cultural and morphological characteristics on YM agar. All fungal isolates were deposited in the Collection of Microorganisms and Cells of the Federal University of Minas Gerais, Brazil, under the code UFMGCB. They are available for use by other scientists.

Fungal identification

The protocol for DNA extraction was described previously in Rosa et al. (2009). For the filamentous fungi, the internal transcribed spacer (ITS) region was amplified with the universal primers ITS1 and ITS4 (White et al. 1990). Amplification of the ITS region was performed as described by Rosa et al. (2009). In addition, amplification of the β-tubulin (Glass and Donaldson 1995) and ribosomal polymerase II genes (RPB2) (Houbraken et al. 2012), which are commonly utilized to fungal taxa with low intraspecific variation, was completed with the Bt2a/Bt2b and RPB2-5F-Pc/RPB2-7CR-Pc 7CR primers, respectively, according to protocols established by Gonçalves et al. (2015). The yeasts were grouped and identified according to protocols established by Kurtzman et al. (2011). Yeast molecular identities were confirmed by sequencing the D1–D2 variable domains of the large-subunit rRNA gene using the primers NL1 and

NL4 as described by Lachance et al. (1999). Yeast isolates with query coverage and identity ≥99% were considered to represent the same taxon. Representative consensus sequences of the fungal taxa were deposited into the GenBank database (Table 1). To achieve species-rank identification based on ITS, β-tubulin data, and ribosomal polymerase II genes (RPB2), the consensus sequence was aligned with all sequences from related species retrieved from the NCBI GenBank database using BLAST (Altschul et al. 1997). Taxa that displayed query coverage and ≤98% identity or an inconclusive taxonomic position were subjected to phylogenetic ITS, β-tubulin and ribosomal polymerase II genes (RPB2)-based analysis for comparison with sequences of *ex type* species deposited in the GenBank database, with estimations conducted using MEGA Version 5.0 (Tamura et al. 2011). The maximum composite likelihood method was employed to estimate evolutionary distances with bootstrap values calculated from 1000 replicate runs. The information about fungal classification generally followed the databases of Kirk et al. (2008), MycoBank (http://www.mycobank.org) and the Index Fungorum (http://www.indexfungorum.org).

Diversity, richness, dominance, and distribution

To quantify the species diversity, richness, and dominance, we used Fisher's α, Margalef's, and Simpson's indices, respectively. All diversity indices were performed using the computer program PAST, version 1.90 (Hammer et al. 2001).

Fungal growth responses to the temperature, pH, and water activity

The fungi were grown on YM at 5, 15, 25, 37, and 45 °C. The fungi were inoculated onto each medium by transferring blocks (4 mm^2) from 10-day-old pre-cultures grown at 15 °C on YM. Plates were incubated in triplicate for 15 days, and the colony diameter was measured in mm. In addition, fragments of the fungal isolates able to grow at 37 and 45 °C were inoculated into YM adjusted to a pH 4, 7, or 9 for 15 days. The experiment was conducted in triplicate, and the colony diameter was measured in mm. Fungi were grown on DG18 ($a_w = 0.95$) and DG36 [36% of glycerol ($a_w = 0.9$)] media. Plates were incubated at 15 °C in

Table 1 Fungal taxa obtained from rocks of continental Antarctica and identified by sequence comparison with the BLASTn match with the NCBI GenBank database

UFMGCB[a]	Density (CFU g^{-1})	Top BLAST search results (GenBank accession number)	Query cover (%)	Identity (%)	No. of bp analyzed	Proposed taxa[f] (GenBank acc. no.)
10039[b,c,d]	250	*Penicillium chrysogenum* (NR077145)	100	100	488	*Penicillium chrysogenum* (KU880718[g], KT749870[h], KT749872[i])
10005[b,c]	185.7	*Penicillium rubens* (NR111815)	100	100	418	*Penicillium tardochrysogenum* (KU880719[g], KT779287[h])
9999[b]	177.7	*Cladosporium halotolerans* (NR119605)	100	99	435	*Cladosporium halotolerans* (KU880720[g])
10034[b]	133.3	*Acremonium egyptiacum* (FN706550)	99	99	487	*Acremonium* sp. (KU880721[g])
AN01AYM1[e]	131.2	*Debaryomyces hansenii* (NG042634)	100	100	498	*D. hansenii* (KU880726[j])
10048[b]	100	*Cladosporium phaenocomae* (NR119950)	100	100	397	*Cladosporium* sp. (KU880722[g])
10017[b,c]	100	*Penicillium citrinum* (NR121224)	100	100	446	*Penicillium citrinum* (KU880723[g], KT749871[h])
10021[b]	100	*Penicillium coffeae* (NR121312)	100	98	478	*Penicillium* cf. *coffeae* (KU880724[g])
AN06ADG1[e]	100	*Rhodotorula mucilaginosa* (AF040632)	100	100	509	*R. mucilaginosa* (KU880727[j])
10044[b]	100	*Talaromyces spectabilis* (AY753330)	95	100	493	*Byssochlamys spectabilis* (KU880725[g])

[a] UFMGCB = Culture of Microorganisms and Cells from the Federal University of Minas Gerais. Taxa subjected to phylogenetic analysis based on the [b] ITS, [c] β-tubulin, [d] Polymerase II and [e] D1/D2 regions for elucidation of taxonomic positions. [f] Taxonomic position suggested by the phylogenetic analyses according to [b] ITS, [c] β-tubulin, and/or [d] Polymerase II. [g] ITS, [h] β-tubulin, [i] Polymerase II, and [j] D1/D2 sequences deposited

triplicate for 15 days, and the colony diameter was measured in mm.

Haemolytic activity, spore size, and dimorphic capability

Haemolytic activity was determined in Sabouraud agar medium (Himedia, India) supplemented with 3% glucose, 7% sheep blood, and a chloramphenicol (Sigma, USA) concentration of 200 μg mL^{-1} (Schaufuss et al. 2007). Petri dishes were incubated at 25, 37, and 45 °C for 7 days. The haemolysis activity was classified as absent, partial, or total. In addition, fragments of the fungal isolates able to grow at 37 and 45 °C were inoculated into YM and subsequently incubated for 15 days in the dark at 15, 37, or 45 °C to determine the spore sizes and polymorphism [the ability to switch between a yeast-like form and a filamentous form (Sánchez-Martínez and Pérez-Martín 2001)] using an optical microscope (Leica DFC425, USA). The experiment was completed in triplicate.

Minimal inhibition concentration (MIC) of antifungal drugs

All fungi able to grow at 37 °C and those that showed haemolytic activity and polymorphism capability were subjected to the minimal inhibition concentration (MIC) determination assay against the antifungal drugs itraconazole and amphotericin B (both Sigma, USA). The MIC protocol was performed using a modified version of methods for filamentous fungi (CLSI M38-A2, vol. 22, no 16). All selected fungi were grown at 25 °C in YM media. After 7–10 days, the spores of each fungus were adjusted to 1–5 × 10^4 spores mL^{-1} in RPMI 1640 media, using 96-well microtiter plates. The fungal spores were submitted to a concentration of amphotericin B from 0.031 to 16 μg mL^{-1} and itraconazole from 0.015 to 8 μg mL^{-1}. Microtiter plates containing the spores were incubated at 37 °C for 48 h. All MIC assays were performed in duplicate.

Macro- and micro-polymorphism morphological characterization

Fungal macroscopic parameters (colony colour and texture, reverse colour, border type, and radial growth rate) and colony diameters were observed on YM medium. Fungal isolates were inoculated into 3-point cultures and incubated for 7 and 15 days at 15 and 37 °C. A duplicate set of MEA plates was incubated at same conditions to determine the microscopic parameters (hyphae, conidiophores, and conidia) using slide cultures mounted in methyl blue in polyvinyl lactophenol and viewed with microscope Leica DM750, Germany.

For scanning electron microscopy, the MA plates were inoculated with a 5 mm mycelial disk from fresh fungal cultures. The cultures were incubated at 15 and 37 °C, and after 7 days, agar disks with fungi were examined by SEM. Specimens were fixed in 2% glutaraldehyde in 0.1 M $NaPO_4$ buffer and washed in buffered 1% OsO_4 for 2 h. The material was dehydrated using an ethanol series (10, 25, 40, 60, 75, 85, 95, and 100%) for 15 min per concentration. The material was dried in a critical point drying apparatus, sputter-coated with gold, and viewed with a FEI Quanta 200 SEM, USA.

Results

Rock characterization

Two types of rocks were obtained, and these were identified as quartzite and phyllite (Suppl. Figs. 2 and 3). The quartzite (Suppl. Figs. 2A and 2B) was classified as *stricto* sensu (*ss*) quartzite or sericite-quartzite. Macroscopically, both have a whitish green colour, fine-to-medium grain size, an oriented microcrystalline texture and a low metamorphic degree. Microscopically, *ss*-quartzite is composed of quartz (40–50%), feldspar (20–30%), sericite (20–30%), carbonate (<5%), epidote (<3%), clinozoizite, and/or perovskita and/or fluorite (<2%). The main difference of sericite-quartzite in relation to *ss*-quartzite is the increased participation of sericite in its mineralogical composition, which includes quartz (35–40%), sericite (30–40%), microcline (<15%), opaque minerals (<10%), epidote (<5%), clinozoizite (<5%), fluorite (<3%), and carbonate (<2%). Minerals are arranged in granolepidoblastic or granoblastic textures, with fine grains averaging from 0.05 to 1.2 mm. Both have prismatic quartz with undulating extinction and trigonal contacts. Sericites occur around the quartz grains in *ss*-quartzite. In sericite-quartzite, the crystals are oriented following the foliation defined by sericite. The feldspar has perthite and myrmekite (quartz with vermiform habit) inclusions. Crusts were observed in the exposed surfaces in some samples of sericite-quartzite (Suppl. Fig. 2C). These crusts have a carbonate composition (Suppl. Fig. 2D), with calcite as the main mineral species. Abrupt contact with the surface, the presence of the concentric layers, and the low carbonate content in the rock indicate that the crusts are formed by secondary carbonate accumulation associated with a re-precipitation in an arid environment.

The phyllite (Suppl. Figs. 3A and 3B) has grey colour, a leafed microcrystalline texture, and a low metamorphic degree. The granulation is thin (<1.0 mm), with sizes ranging between 0.5 and 1.0 mm. The minerals are muscovite (>50%), quartz (<25%), orthoclase (<7%), plagioclase (<7%), carbonate, fluorite, chlorite, opaque (<3% for all),

and epidote (<2%). The mineralogical composition allows the rock to be classified as a muscovite philite. The texture at the microscopic level was lepidogranoblastic and inequigranular. The phyllite shows foliation defined by oriented mica and elongated quartz and fractures that are likely associated with the freeze–thaw process (Suppl. Figs. 3B and 3D). Part of the feldspar is altered by sericitization, and some orthoclases have perthite inclusions. In addition, porphyroclasts of stretched quartz were observed. The phyllite samples contain dissolution cavities, indicating differential mineral weathering (Suppl. Figs. 3C and 3E). These cavities are partially filled by the secondary materials rich in iron (Suppl. Fig. 4A) or aluminium (Suppl. Fig. 4B). These features suggest the existence of geochemical modification processes, such as fungal weathering processes (Burford et al. 2003a, b; Gadd 2007).

Fungal taxonomy and diversity

Seventy fungal isolates were obtained and identified as species of the genera *Acremonium Byssochlamys*, *Cladosporium*, *Debaryomyces*, *Penicillium,* and *Rhodotorula* using molecular techniques (Table 1; Suppl. Figs. 5, 6, 7). The fungal colony density present in the rock samples ranged from 100 to >300 CFU g^{-1} among the different culture media. *Cladosporium halotolerans*, *Penicillium chrysogenum,* and *Penicillium tardochrysogenum* were the most frequently identified species, which occurred on at least three culture media and displayed the highest CFU g^{-1} (Suppl. Table 1). In contrast, *Cladosporium* sp. *Penicillium* cf. *coffeae*, *Rhodotorula mucilaginosa,* and *Byssochlamys spectabilis* were rare taxa within the fungal community. The diversity, richness, and dominance were variable in the fungal community inhabiting continental Antarctic rocks (Table 2). As expected, the fungal community present in the Antarctic rocks showed lower diversity and richness but high dominance values.

Virulence factors

The fungal isolates associated with were able to grow at different temperatures and demonstrated haemolytic activities at 25 and 37 °C (Suppl. Table 3). *Acremonium* sp., *B. spectabilis*, *D. hansenii*, *P. chrysogenum*, *P. citrinum*, *P. tardochrysogenum,* and *R. Mucilaginosa* displayed mesophilic behaviour, growing from 15 to 37 °C. Only *Cladosporium* sp., *P. chrysogenum,* and *P. tardochrysogenum* could grow at 5 °C.

Fifty-three fungal isolates grown on the DG18 medium showed xerophilic behaviour (Suppl. Table 2). These included *Cladosporium halotolerans*, *Cladosporium* sp., *Penicillium chrysogenum*, *Penicillium citrinum*, *Penicillium* cf. *coffeae*, and *Penicillium tardochrysogenum* grown on DG36 medium, all of which had the physiological ability to survive in xerophilic conditions.

Table 2 Ecological indices of the fungal communities obtained from quartzite and phyllite rocks from continental Antarctica

Rock type	Ecological indices			
	No. of fungal taxa	Fisher α	Margalef	Simpson's
Quartzite	6	0.85	0.73	0.76
Phyllite	8	1.16	0.99	0.86

Different isolates of *D. hansenii*, *P. chrysogenum*, *P. citrinum*, *P. tardochrysogenum,* as well as the taxa *Cladosporium* sp., *P.* cf. *coffeae*, and *R. mucilaginosa*, represented by only one isolate, were able to produce partial haemolysis at 25 and/or 37 °C (Suppl. Table 3). However, 31 isolates of *P. chrysogenum*, *P. citrinum,* and *P. tardochrysogenum* displayed mycelial growth and haemolytic activity at 37 °C. In addition, *Acremonium* sp., *P. citrinum,* and *P. tardochrysogenum* showed spore sizes ranging from 2.81 to 5.13 µm diameter at 37 °C (Suppl. Table 4). In contrast, *P. tardochrysogenum* UFMGCB 10030 (Suppl. Fig. 8) and *P. chrysogenum* UFMGCB 10040 (Suppl. Fig. 9) displayed macro- and micromorphological culture polymorphisms when cultivate at different temperatures. *P. tardochrysogenum* and *P. chrysogenum* showed at 15 °C normal conidiophores, conidiogenic cells, and conidia; however, at 37 °C, both fungi showed yeast-like growth with the evident chlamydospores structures. Isolates of *Acremonium* sp., *D. hansenii*, *P. citrinum*, *P. tardochrysogenum,* and *Rh. mucilaginosa* were able to grow at pH levels of 4, 7 and 9 at 37 °C (Suppl. Table 5). An interesting result was the growth of the majority of *P. chrysogenum* isolates at pH 9 at 37 °C. Only *B. spectabilis* was capable of growing at a pH of 4, 7, and 9 at 37 as well as 45 °C.

All fungi able to grow at 37 °C, with haemolysis and dimorphic capability, were subjected to evaluation of their resistance to the antifungal drugs amphotericin B and itraconazole (Table 3). Twenty-one isolates of *P. chrysogenum* and *P. tardochrysogenum* showed different resistance patterns against the antifungal drugs. Among them, four isolates of *P. chrysogenum* and two of *P. tardochrysogenum* were resistant to amphotericin B and/or itraconazole at concentrations of ≥4 µg mL^{-1}.

Discussion

Despite the extreme conditions of continental Antarctica, the fungal communities associated with rocks were dominated by Eurotiomycetes taxa (*Penicillium* and *Byssochlamys*), followed by Sordariomycetes classes (*Acremonium*), Dothideomycetes (*Cladosporium*),

Table 3 Rate of growth in different temperatures (°C), hemolytic activity, spore size, polymorphic capability, and drug sensibility against antifungal agents

UFMGCB[a]	Fungal species	Fungal growth (mm)[b] at 37 °C	Hemolytic activity		Spores size µm (length × width)[b] at temperature in °C		Polymorphic capability	Drug sensibility (MIC µg mL^{-1}) at 37 °C	
			25 °C	37 °C	15 °C	35 °C		Amphotericin B	Itraconazole
10003	*Penicillium chrysogenum*	6 ± 1	Ph	Ph	2.42 ± 0.78	–	+	0.25	0.25
10023	*P. chrysogenum*	7.7 ± 0.6	Ph	Ph	3.24 ± 0.75	–	+	0.125	0.5
10024	*P. chrysogenum*	8.3 ± 0.6	Ph	Ph	3.16 ± 0.36	–	+	0.5	0.25
10027	*P. chrysogenum*	7.3 ± 1.2	Ah	Ph	3.14 ± 0.23	–	+	0.5	0.5
10028	*P. chrysogenum*	7.0 ± 1.7	Ah	Ph	3.07 ± 0.34	–	+	0.125	0.5
10029	*P. chrysogenum*	6.3 ± 0.6	Ph	Ph	3.49 ± 0.44	–	+	0.5	0.5
10037	*P. chrysogenum*	3.3 ± 5.8	Ph	Ph	2.82 ± 0.14	–	+	2.0	0.5
10038	*P. chrysogenum*	8.7 ± 3.8	Ph	Ph	3.06 ± 0.16	–	+	2.0	0.5
10039	*P. chrysogenum*	12 ± 4.4	Ph	Ph	3.22 ± 0.39	–	+	2.0	0.5
10040	*P. chrysogenum*	12.7 ± 2.1	Ph	Ph	3.18 ± 0.43	–	+	4.0	0.5
10042	*P. chrysogenum*	14 ± 1.7	Ph	Ph	2.69 ± 1.00	–	+	2.0	0.5
10049	*P. chrysogenum*	7.7 ± 5	Ah	Ph	2.81 ± 0.47	–	+	1.0	<0.0015
10000	*P. tardochrysogenum*	4.3 ± 0.6	Ph	Ph	2.23 ± 1.29	3.20 ± 0.16	+	1.0	0.5
10005	*P. tardochrysogenum*	5 ± 2.6	Ph	Ph	2.88 ± 0.61	–	+	0.5	0.25
10013	*P. tardochrysogenum*	6.3 ± 1.5	Ph	Ph	3.06 ± 0.40	5.13 ± 0.84	+	2.0	0.5
10018	*P. tardochrysogenum*	7 ± 1	Ph	Ph	3.57 ± 0.50	3.00 ± 0.26	+	1.0	0.25
10019	*P. tardochrysogenum*	7.3 ± 1.2	Ph	Ph	3.09 ± 0.45	–	+	2.0	0.5
10022	*P. tardochrysogenum*	6.7 ± 2.1	Ph	Ph	3.00 ± 0.75	3.39 ± 0.43	+	2.0	0.5
10025	*P. tardochrysogenum*	5.3 ± 0.6	Ph	Ph	3.44 ± 0.40	–	+	2.0	0.5
10026	*P. tardochrysogenum*	5.3 ± 0.6	Ph	Ph	3.37 ± 0.37	–	+	2.0	0.5
10030	*P. tardochrysogenum*	6.3 ± 0.6	Ph	Ph	3.02 ± 0.25	–	+	4.0	0.5

–, absence of growth; +, growth; Ph, partial hemolysis; Ah, absence of hemolysis

[a] UFMGCB = Culture of Microorganisms and Cells from the Federal University of Minas Gerais, Brazil

[b] Average colonies diameter/standard deviation (mm)

Saccharomycetes (*Debaryomyces*), and Microbotryomycetes (*Rhodotorula*). We recovered several fungal isolates from the Antarctic rocks; however, despite the use of petrographic analysis to check the evidences of fungal growth inside the rocks, we assumed that some spores, hypha fragments, or resistance structures might be recovered. Studies able to show the growth activity of mycelial endolithic microorganisms inside the rocks are complicated, because these organisms live close to the limit of their physiological adaptability, which have very slow growth. According to Wierzchos et al. (2004), endolithic microorganisms, such as those present in Antarctica, can occur as dormant form along with non-viable cells that retain their morphological integrity and Mulyukin et al. (2002) reported these cells as micromummies. To detect the presence of fungi inside the continental Antarctic rocks, we used the SEM microscopy, which is able to show the direct or indirect presence of the fungi in the rocks. However, from the perspective of the studies of mycological ecology in the extreme environmental conditions of Antarctica, further detailed studies will be necessary to verify if these endolithic fungi have active growth inside the rocks.

Four *Penicillium* species (Euromycetes) were identified in the Antarctic rocks. Among them, *P. chrysogenum* and *P. tardochrysogenum* showed the highest densities. According to Tang and Lian (2012), *Penicillium* species appear to be ubiquitous in endolithic rock environments. *Penicillium chrysogenum* is a very common pan-global species found in indoor environments, deserts, dried foods, salterns, and cheese (Frisvad and Samson 2004). In Antarctica, *P. chrysogenum* was detected in different samples, including soil with plant populations, ornithogenic soils (McRae et al. 1999), and oligotrophic continental soils (Godinho et al. 2015). This species can also be associated with macroalgae (Godinho et al. 2013). Zucconi et al. (2012) isolated *P.*

chrysogenum as a dominant species present in permafrost in Antarctica. *Penicillium tardochrysogenum* is considered endemic to Antarctica and was isolated from the soil of the McMurdo Dry Valley (Houbraken et al. 2012).

Some *Cladosporium* species are isolated from extreme environments, including hypersaline habitats (Gunde-Cimerman et al. 2000). The sequences of the nine isolates obtained from the Antarctic rocks displayed high similarity levels (99–100%) with sequence of the species *Cladosporium halotolerans* CBS119416 (NR119605), which were isolated from hypersaline environments. According to Zalar et al. (2007), *C. halotolerans* has been detected in hypersaline water of salterns and other saline environments. It is, therefore, possible that this fungus is a species closely linked to salty or hypersaline environments, although additional sampling is necessary for this to be proven.

We obtained three isolates of *Acremonium* able to grow in the 15–37 °C temperature range. The polyphyletic genus *Acremonium* shelters approximately 117 species, including saprobes, endophytes, and phytopathogens species (Kirk et al. 2008). Different *Acremonium* species have been obtained from Antarctica from seawater, plants, lichens, and soil-dwelling invertebrates (Pugh and Allsopp 1982; Zucconi et al. 1996; Möller and Dreyfuss 1996; Bridge and Denton 2007).

Only two yeast species (*D. hansenii* and *R. mucilaginosa*) were isolated from the continental Antarctic rocks. *Debaryomyces hansenii* is a cryotolerant and halotolerant ascomycetous yeast associated with food spoilage (Kurtzman et al. 2011) that was isolated from the soil of continental and peninsular Antarctica (Arenz and Blanchette 2011; Godinho et al. 2015). *Rhodotorula mucilaginosa* is a cosmopolitan yeast species present in substrates of terrestrial and aquatic (freshwater and marine) habitats, including those with extreme conditions (Kurtzman et al. 2011). In Antarctica, *R. mucilaginosa* was found in soil (Ray et al. 1989) and lakes (Vaz et al. 2011; Gonçalves et al. 2012) and associated with macroalgae (Loque et al. 2010; Godinho et al. 2013; Furbino et al. 2014) and lichens (Santiago et al. 2015).

The physiological temperature experiment showed that the isolate *Byssochlamys spectabilis* UFMGCB 10044 was obtained from the continental Antarctic rocks grown between 15 and 45 °C. *Byssochlamys spectabilis* [also described as *Talaromyces spectabilis* by Udagawa and Suzuki (1994)] is a teleomorph of *Paecilomyces variotii* (Houbraken et al. 2008). As a synonymous species to *P. variotii*, *B. spectabilis* was reported as a cosmopolitan fungus present in soils and indoor environments. It is associated with plants, animals, and foodstuffs (Samson 1974; Samson et al. 2004). However, this is the first recorded evidence that this genus is native to the Antarctica region.

Virulence factors are the properties that increase the survival, growth, and propagation of fungi in animal tissues. The main virulence factors include the ability to grow at 37 °C, neutral pH, and xerophilic conditions (de Hoog et al. 2001, 2005), haemolytic activity (Luo et al. 2001), spore size ≤5 μm (Latge 2001) dimorphic activities (Klein and Tebbets 2007), and resistance to antifungal drugs (Kanafani and Perfect 2008), which may indicate the capability of the fungi to act as opportunistic agent causing diseases. All RIF isolates recovered from the continental Antarctic rocks displayed different virulence factors. Indeed, various isolates were able to grow between 37 until 45 °C and produce partial haemolysis on blood agar medium at 37 °C.

In addition, several isolates of *P. chrysogenum* and *P. tardochrysogenum* showed spore sizes ranging from ≤4 μm of diameter at 37 °C, with morphological culture polymorphism at 15 and 37 °C. Few fungi have the capability to change morphology from yeast to hyphae and contrariwise during their lifecycle. According to Gauthier (2015), these pathogens can be classified as thermal (morphologic switch induced by temperature) and non-thermal dimorphic fungi. The dimorphic fungi (as species of the genera *Blastomyces*, *Coccidioides*, *Histoplasma*, *Paracoccidioides*, *Sporothrix,* and *Penicillium*) grow as a mold in soil at ambient temperature and change to yeast after infectious spores are inhaled into the lungs of a mammalian host, often at 37 °C (Klein and Tebbets 2007). For mammalian fungi, the transition of mycelial form to a yeast or yeast-like growth results in production of proteins to evade immune defenses or toxins to alter host behaviour; in addition, growth as yeast coupled with thermotolerance allows for replication within mammalian phagocytic cells, which promotes dissemination Gauthier (2015). These wild fungal taxa, obtained from an extreme Antarctic environment, may be useful models to further studies about fungal polymorphism in vitro and in vivo models.

Despite we recover isolates of *P. chrysogenum* with different physiological virulence factors in vitro, according to de Hoog et al. (2000), the majority of the *Penicillium* species are mesophilic fungi that can barely grow at 37 °C, but unable to be etiologic agents of systemic disease. However, known ubiquitous species like *P. chrysogenum*, *P. citrinum,* and *P. decumbens* present limited or optimal growth at 37 °C, which have been reported as opportunistic fungi in immunocompromised patients (de Hoog et al. 2000). *Penicillium chrysogenum* was reported to cause otomycoses, endophthalmitis, keratitis, endocarditis, cutaneous infection, and systemic infection in immunocompromised patients (de Hoog et al. 2001). In addition, *P. chrysogenum* was reported to cause a fungal bloodstream infection in an immunocompromised patient (Swoboda-Kopec et al. 2002) and cerebral disease in a healthy patient (Lyratzopoulos et al. 2002). *Penicillium citrinum* was reported as the causative agent of urinary tract infection, lung infection, and keratitis (de Hoog et al. 2001). Mok et al. (1997) reported a

fatal infection in a leukaemia patient caused by *P. citrinum*. Several isolates of *P. tardochrysogenum* obtained from the Antarctic rocks were able to produce haemolytic activity at 37 °C and growth at a pH of 9, with xerophilic behaviour, a spore diameter size between 3.0 and 5.13 µm and dimorphic capability. Despite representing a recently described new species considered endemic to Antarctica, *P. tardochrysogenum* was reported to be a producer of the extracellular secondary metabolites, defined as extrolites, such as penicillins, secalonic acids D & F, asperentins, and the uncharacterized extrolite met Ø (Houbraken et al. 2012), which might explain its capability to produce haemolytic activity. Usually, fungi able with haemolytic activity are reported as producer of mycotoxins (Greenhill et al. 2008).

We also identified the taxa *Cladosporium* sp. and C. *halotolerans* in the rock samples, which presented different virulence factors. *Cladosporium* species are ubiquitous, saprobic dematiaceous fungi, which very rarely cause infections in humans (de Hoog et al. 2001). *Cladosporium* is often associated with allergic rhinitis or on superficial or in deep lesions (Drabick et al. 1990; Gugnani et al. 2000, 2006; Sang et al. 2012) but rarely can cause disseminated infections (Kantarcioğlu et al. 2002; Kantarcıoğlu and de Hoog 2004; de Hoog et al. 2011; Lalueza et al. 2011). Sandoval-Denis et al. (2015) studied the molecular taxonomy of different *Cladosporium* isolates recovered from clinical samples to determine if some could possibly be rare potential pathogens. Among them, the authors identified *C. halotolerans* as the most frequent taxa, which was isolated from human bone marrow, bronchi, lymph node, catheter tip, nasal, scalp, and toenail samples, as well as the dermis of marine mammals. However, as mycosis caused by *Cladosporium* species was poorly reported, just the recover to these taxa from continental Antarctic rocks as well as their capability to present physiological virulence factors in vitro do not imply that they cause infection in humans. Further detailed studies will be necessary to detect if these taxa can be opportunistic pathogenic fungi to humans.

However, according to Guarro et al. (1997), some *Acremonium* species are important opportunistic pathogens and may cause invasive disease in humans. According to Perdomo et al. (2011), the most common anatomic sites for *Acremonium* isolates were the respiratory tract (41.3%), nails (10.7%), and the eye (9.3%). The sequences of *Acremonium* isolates recovered from Antarctic rocks displayed phylogenetic affinities with the sequences of *A. egyptiacum* and *A. sclerotigenum*. Strains of *A. egyptiacum* and *A. sclerotigenum* have been reported from different human infections, including from blood, tracheal aspirate, sinus, and toenails (Perdomo et al. 2011).

We obtained only two yeast species (*D. hansenii* and *Rh. mucilaginosa*) from the Antarctic rocks, which showed some virulence factors. Despite not representing a common human pathogen, the ubiquitous yeast *D. hansenii* (teleomorph of *Candida famata*) has been described in human infections, mainly associated with catheter-related bloodstream infection (Desnos-Ollivier et al. 2008). Kurtzman et al. (2011) reported that *R. mucilaginosa* has been collected from human sources (also with the name *Rh. rubra*).

Byssochlamys spectabilis is a thermotolerant species able to grow quickly, produce mycotoxins, and survive at heat treatments at low oxygen levels; these characteristics make *B. spectabilis* an organism usually found in foodstuffs (Houbraken et al. 2008). In addition, *B. spectabilis* seems to cause different human infections, such as endocarditis (Castro et al. 1990).

The ecological indices of Fisher α, Margalef, and Simpson varied according to the rock type range in two different types of rocks (Table 2). The Fisher α index was higher in the phyllites, which presented varied mineral degradation features, suggesting greater weathering (Suppl. Figs. 3 and 4). The lowest Fisher α value was observed in the coarse-grained quartzites with calcite crust (Suppl. Fig. 2). Ca enrichment at the surface represented a resistance and drier microenvironment in the quartzites; in addition, the closed voids and the formation of hydrophobic crusts are less favourable to biological colonization. Conversely, the finer (pellitic) matrix of phyllites contributed to greater microporosity, resulting in higher water retention. In addition, increasing mica in relation to quartz might have contributed to the higher nutrient content of the rocks. According to Burford et al. (2003a, b), fungal growth in these microenvironments promotes biomechanical and biochemical degradation of minerals, especially in fine particles; this phenomenon was observed in the rocks of the Atacama Desert, as published by Gonçalves et al. (2015). Our results are consistent with those of Gadd (2007), who proposed that fungal weathering processes may contribute to the dissolution, sorption, transport, diffusion, and recrystallization of mobilized cations. In fact, the cations formed the filling-material observed in some cavities of the phyllite samples.

Conclusion

To our knowledge, the present study represents the first report detailing the opportunistic pathogenic virulence potential of fungi associated with rocks from the desert of continental Antarctica. The fungi obtained were phylogenetically close to opportunistic pathogenic and mycotoxigenic taxa and showed one or more potential physiologic virulence characteristics in vitro. Our results suggest that these taxa may represent primitive eukaryotic organisms isolated geographically with genomes that allow studying

the evolutionary origins of opportunistic virulence in fungi, in special to those able to cause diseases in immunocompromised persons, compared with their pathogenic and/or mycotoxigenic relatives. However, further studies will be necessary to affirm if these fungi are or not a risk to humans, mainly due the possible effects of the climate global changes in Antarctica, which might release "primitive" and/or unknown microbes to out of Antarctica. In addition, the presence of different fungal isolates within rocks in the ultra-extreme cold and dry environment of the Ellsworth, coupled with the observed chemical alteration processes, suggests that endolithic domains may represent a favourable microhabitat able to support microbial life better than the extreme conditions of the outside environment.

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Compliance with ethical standards

Conflict of interest The authors reported no conflict of interest.

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