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ENVIRONMENTAL MICROBIOLOGY - RESEARCH PAPER

Pathogenic potential of an environmental *Aspergillus fumigatus* strain recovered from soil of *Pygoscelis papua* (Gentoo penguins) colony in Antarctica

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Abstract

Aspergillus fumigatus is a common opportunistic pathogen in different animals, including birds such as penguins. For the first time, a fungal strain identified as *A. fumigatus* was isolated from soil in the nests of gentoo penguins, *Pygoscelis papua*, on Livingston Island, South Shetland Islands (maritime Antarctica). This isolate (*A. fumigatus* UFMGCB 11829) displayed a series of potentially pathogenic characteristics in vitro. We evaluated its detailed molecular taxonomy and submitted the *A. fumigatus* UFMGCB 11829 Antarctic strain to in vivo pathogenic modelling. The isolate was confirmed to represent *A. fumigatus* morphological and phylogenetic analysis showed that it was closely related to *A. fumigatus* sequences reported from animals, immunosuppressed humans, storage grains, plants and soils. The strain displayed the best mycelial growth and conidia production at 37 ºC; however, it was also able to grow and produce conidia at 15º, demonstrating its capability to survive and colonize penguin nest at least in the summer season in maritime Antarctica. In pathogenicity tests, healthy mice did not showed symptoms of infection; however, 50% lethality was observed in immunosuppressed mice that were inoculated with 10^6 and 10^7 spores. Lethality increased to 100% when inoculated with 10^8 spores. Our data highlight the potential pathogenicity of opportunistic *A. fumigatus* that may be present in the Antarctic, and the risks of both their further transfer within Antarctica and outwards to other continents, risks which may be exacerbated due global climatic changes.

Keywords Antarctica · Extremophiles · Pathogenic · Penguin · Opportunistic

Introduction

The fungal genus *Aspergillus* includes many widely distributed and free-living species, which perform an important component of global carbon and nitrogen recycling. These fungi produce tiny hydrophobic conidia that can easily enter and disperse in the air column, as well as being able to survive for extended periods in different substrates and under varied environmental conditions [1]. *Aspergillus fumigatus* is a common opportunistic fungal pathogen able to affect different birds and other animals, including captive penguins [2]. It is also capable of infecting immunocompromised humans and is able to tolerate the human body temperature of ~ 37 °C. The mechanism of infection by *A. fumigatus* is generally via airborne conidia inhalation, followed by deposition in the bronchioles or alveolar spaces. Once within the body (especially in the lungs), the fungus exhibits several defense mechanisms that assist it to escape the response of the body's

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macrophages, including conidial resistance, melanin production and antioxidant activity [1]. *Aspergillus fumigatus* also causes allergic bronchopulmonary aspergillosis (ABPA), which is recognized as a serious disease affecting asthmatic patients [3, 4].

Antarctica represents one of the last pristine regions on the Earth, whose unique biodiversity, dominated by microbes, exhibits well developed resistance to extreme conditions such as low temperatures, desiccation, high solar radiation, a lack of available nutrients and strong winds [5]. The diversity of fungi present in Antarctica is very high, comprising complex communities of decomposer (saprophytic), mutualistic and parasitic species [6]. The most important functional roles of fungi in Antarctica relate to their activities in decomposing and recycling organic matter and to their mutualistic relationships (e.g. symbiosis with algae in lichens) with other Antarctic organisms. Studies have also shown that a minority of Antarctic fungi are opportunistic and potential plant and animal pathogenic fungi [5, 7–10]. These deserve special attention due the potential reactivation of dormant fungi as a consequence of regional climate changes, a threat of particular relevance in Antarctica Peninsula region. Recently, an important alert was presented by da Silva et al. [11], who demonstrated that Antarctic permafrost soil, an ecosystem containing many dormant but viable organisms and propagules and also most directly threatened by warming leading to permafrost thaw, harbors viable fungal taxa capable of growth at 37 °C. These taxa include fungi recognized as opportunistic pathogens in humans or animals, such as *Rhodotorula mucilaginosa*, *Aspergillus thermomutatus* and *Penicillium* species, that demonstrated different in vitro and in vivo virulence and pathogenic capabilities.

de Sousa et al. [8] studied opportunistic fungi associated with ornithogenically-influenced soils close to the nesting areas of different Antarctic birds. Among the fungi found, strains identified as *A. fumigatus* were amongst the most abundant taxa recovered, the first formal record of this species in Antarctica. These grew at a range of different pH values, temperatures of 37° and 40 °C, produced spores of ≤ 1 µm diameter and their amphotericin B minimum inhibitory concentration (MIC) varied between 0.5 and 1 µg/mL. The study also noted that some *A. fumigatus* strains, after being subjected to phagocytosis by the amoeba *Acanthamoeba castellanii*, displayed a 5- to tenfold increase in amphotericin B MIC after 2 h of contact. In the current study, we further evaluated the detailed the taxonomy, physiologic and phylogenetic characteristics of Antarctic *A. fumigatus* strain and submitted it to in vivo pathogenic modelling.

Materials and methods

Aspergillus fumigatus origin

The fungal strain *Aspergillus fumigatus* UFMGCB 11829 was originally isolated from soil in the nests of the gentoo penguin, *Pygoscelis papua*, obtained at Hannah Point, Livingston Island (South Shetland Islands, Maritime Antarctic) in December 2013 [8]. For the current study, the strain was obtained from the Collection of Microorganisms and Cells of the Universidade Federal de Minas Gerais, Brazil.

Molecular taxonomy of *Aspergillus fumigatus* UFMGCB 11829

Aspergillus fumigatus UFMGCB 11829 was submitted to taxonomic study. The DNA extraction was previously described by Rosa et al. [12] and the amplification of the internal transcribed spacer (ITS) region was amplified with the universal primers ITS1 and ITS4 [13]. Amplification of the β-tubulin gene [14], commonly utilized in studies of fungal taxa with low intraspecific variation, was completed using the primers Bt2a/Bt2b, following Gonçalves et al. [15]. To achieve species-rank identification based on ITS and β-tubulin data, the consensus sequence was aligned with all sequences from related species retrieved from the NCBI GenBank database using BLAST [16]. The maximum composite likelihood method was employed to estimate evolutionary distances with bootstrap values calculated from 1,000 replicate runs using the evolutionary program MEGA 11 [17].

In vitro morphological plasticity of *Aspergillus fumigatus* UFMGCB 11829 in relation to temperature

Classical morpho-physiological techniques were used to study *A. fumigatus* UFMGCB 11829 in culture under different temperature regimes. The fungus was inoculated into sets of three Petri dishes on the following media: YM agar (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 2% glucose, 2% agar) and Czapec Dox agar (CZ) (Kasvi, Brazil), and incubated for 7 d at 15º, 37° or 50 °C. Macroscopic parameters (colony colour, texture, reverse colour, border type and radial growth rate) and colony diameters (mycelial speed) were observed on the different media as described below. Colony colors follow the classification proposed by Kornerup and Wanscher [18]. To determine microscopic parameters (hyphae, conidiophores and conidia) fungal spots were incubated on CZ medium for 7 d at 15°, 37 or 50 °C using slide cultures then mounted

in lactophenol and viewed under a compound microscope (Leica DM750, Germany). Ten spores were used to measure conidia size (µm) and calculate the standard deviation.

For scanning electron microscopy, the fungus was inoculated on CZ medium and incubated at 15°, 37° or 50 °C for 7 d. The mycelia 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 a graded ethanol series (10, 25, 40, 60, 75, 85, 95 and 100%) for 15 min per concentration. It was then dried in a critical point drying apparatus, sputter-coated with gold, and viewed with a FEI Quanta 200 SEM, USA.

In vivo assay of *Aspergillus fumigatus* UFMGCB 11829 against healthy and immunocompromised BALB C male mice

The in vivo assays were performed with five male BALB/c mice, with six weeks old, as described by Ferreira et al. [19]. Briefly, prior to intratracheal infection, animals were anesthetized by intraperitoneal (i.p.) injection with ketamine hydrochloride (ZOOSERV) (60 mg/kg) and xylazine (ZOOSERV) (10 mg/kg) in sterile saline solution (0.85%). Three different inocula were tested: 1×10^5, 10^6 or 10^7 spores of *A. fumigatus* UFMGCB 11829. A control group was inoculated with phosphate buffered saline (PBS). The mice were monitored daily for survival and symptoms such as piloerection, hyperventilation, reduced motility, weight loss was observed.

Further groups of six mice were immunosuppressed using oral Dexamethasone (Decadron/Aché 10 mg/kg/day) in their drinking water for 4 d prior to the experiment [20]. Then the mice were infected as described above, including 10^8 spores of fungus and were monitored for survival. All experimental procedures were carried out according to the standards of the Brazilian Society of Laboratory Animal Science/Brazilian College for Animal Experimentation (available at http://www.sbcal.org.br). The study was approved by the Ethics Committee in Animal Experimentation of the Universidade Federal de Minas Gerais (CEUA/UFMG; protocol 172/2020).

Results

Taxonomy of *Aspergillus fumigatus* UFMGCB 11829

The study strain was confirmed as *A. fumigatus* species through comparison with the ITS and beta-tubulin sequences of type species deposited in GenBank. Phylogenetic analysis of *A. fumigatus* UFMGCB 11829 using the ITS region showed that the strain was closely related to *A. fumigatus* sequences obtained from multiple sources (Fig. 1), including the type species *A. fumigatus*

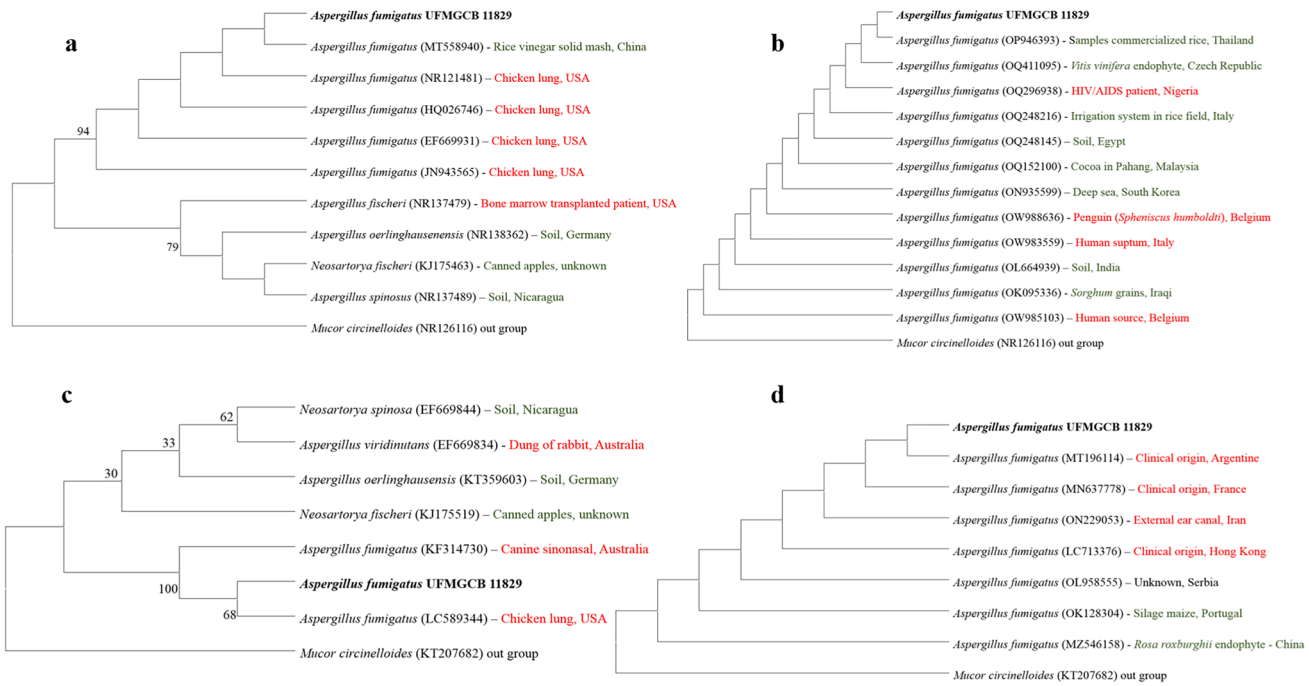

Fig. 1 Phylogenetic analysis of the studied Antarctic strain, *Aspergillus fumigatus* UFMGCB 11829, sequences (in bold) using ITS (**a**) and beta-tubulin (**c**) sequences compared with type, ITS (**b**) and beta-tubulin (**d**) compared with non-type sequences from different origins deposited in the GenBank database

MT558940 (recovered from rice vinegar solid mash), *A. fumigatus* NR121481 (chicken lung origin), and non-type sequences of *A. fumigatus* OP946393 (from commercialized rice), *A. fumigatus* OQ411095 (endophyte of *Vitis vinifera*) and *A. fumigatus* OQ296938 (HIV/AIDS human patient). Phylogenetic analysis of the beta-tubulin region also showed that the sequence of *A. fumigatus* UFMGCB 11829 was closest to those of type sequences of *A. fumigatus* LC589344 (chicken lung), *A. fumigatus* KF314730 (canine sinonasal origin) and non-type sequences of *A. fumigatus* MT196114 (clinical origin) and *A. fumigatus* MN637778 (clinical origin). Detailed information about the multiple origins of the closest non-type sequences of *A. fumigatus* is given in Table 1.

In vitro temperature plasticity of *Aspergillus fumigatus* UFMGCB 11829

In a physiological assay, *A. fumigatus* strain UFMGCB 11829 displayed the best mycelial growth and conidia production at 37 and 50 ºC, respectively. However, the fungus also was able to grow and produce conidia at 15 ºC, thereby demonstrating considerable temperature plasticity. Full macro- and micromorphological characteristics are presented in Suppl. Table 1 and Fig. 2.

In vivo assay using healthy and immunocompromised BALB C male mice

In general, healthy mice did not present any symptoms (piloerection, hyperventilation, reduced motility and weight loss) related to infection, which was corroborated by the absence of lethality (Fig. 3a, b). However, mice showed significant weight loss around the seventh day after infection with 10^5 and 10^7spores. Weight variation differed in immunosuppressed mice, with 50% survival when inocula of 10^6 and 10^7 spores were tested, and 100% lethality with inoculum of 10^8 spores (Fig. 3c, d).

Discussion

Taxonomy and phylogenetic analysis of *Aspergillus fumigatus* UFMGCB 11829

Aspergillus is a widely distributed fungal genus that includes species with varied ecological and functional characteristics, including some species recognized as important opportunist pathogens able to affect animals and humans [1]. Opportunistic *Aspergillus* species produce tiny hydrophobic conidia that are able to enter and disperse easily in the air column and that have extended survival under various environmental

Table 1 Data relating to non-type sequences of *Aspergillus fumigatus* strains deposited in the GenBank database phylogenetically closest to *Aspergillus fumigatus* UFMGCB 11829

Origin	DNA region	Query cover (%)	Identity (%)	GenBank sequence ID	Origin	Reference
Clinical	ITS[a]	100	100	OQ296938	PHIV/AIDS patient, Nigeria	Unpublished
		100	100	OW988636	Penguin (*Spheniscus humboldti*), Belgium	Unpublished
		100	100	OW983559	Human sputum, Italy	Unpublished
		100	100	OW985103	Human source, Belgium	Unpublished
	BT[b]	100	100	MT196114	Clinical origin, Argentina	[21]
		100	100	MN637778	Clinical origin, France	[21]
		100	100	ON229053	External ear canal, Iran	Unpublished
		100	100	LC713376	Clinical origin, Hong Kong	Unpublished
Non-clinical	ITS	100	100	OP946393	Samples of commercialized rice, Thailand	[22]
		100	100	OQ411095	*Vitis vinifera* endophyte, Czech Republic	Unpublished
		100	100	OQ248216	Irrigation system in rice field, Italy	Unpublished
		100	100	OQ248145	Soil, Egypt	[23]
		100	100	OQ152100	Cocoa in Pahang, Malaysia	Unpublished
		100	100	ON935599	Deep sea, South Korea	Unpublished
		100	100	OL664939	Soil, India	Unpublished
		100	100	OK095336	*Sorghum* grains, Iraq	Unpublished
	BT	100	100	OL958555	Unknown, Serbia	Unpublished
		100	100	OK128304	Silage maize, Portugal	Unpublished
		100	100	MZ546158	*Rosa roxburghii* endophyte, China	Unpublished

[a]Internal Transcribed Spacer. [b]beta-tubulin

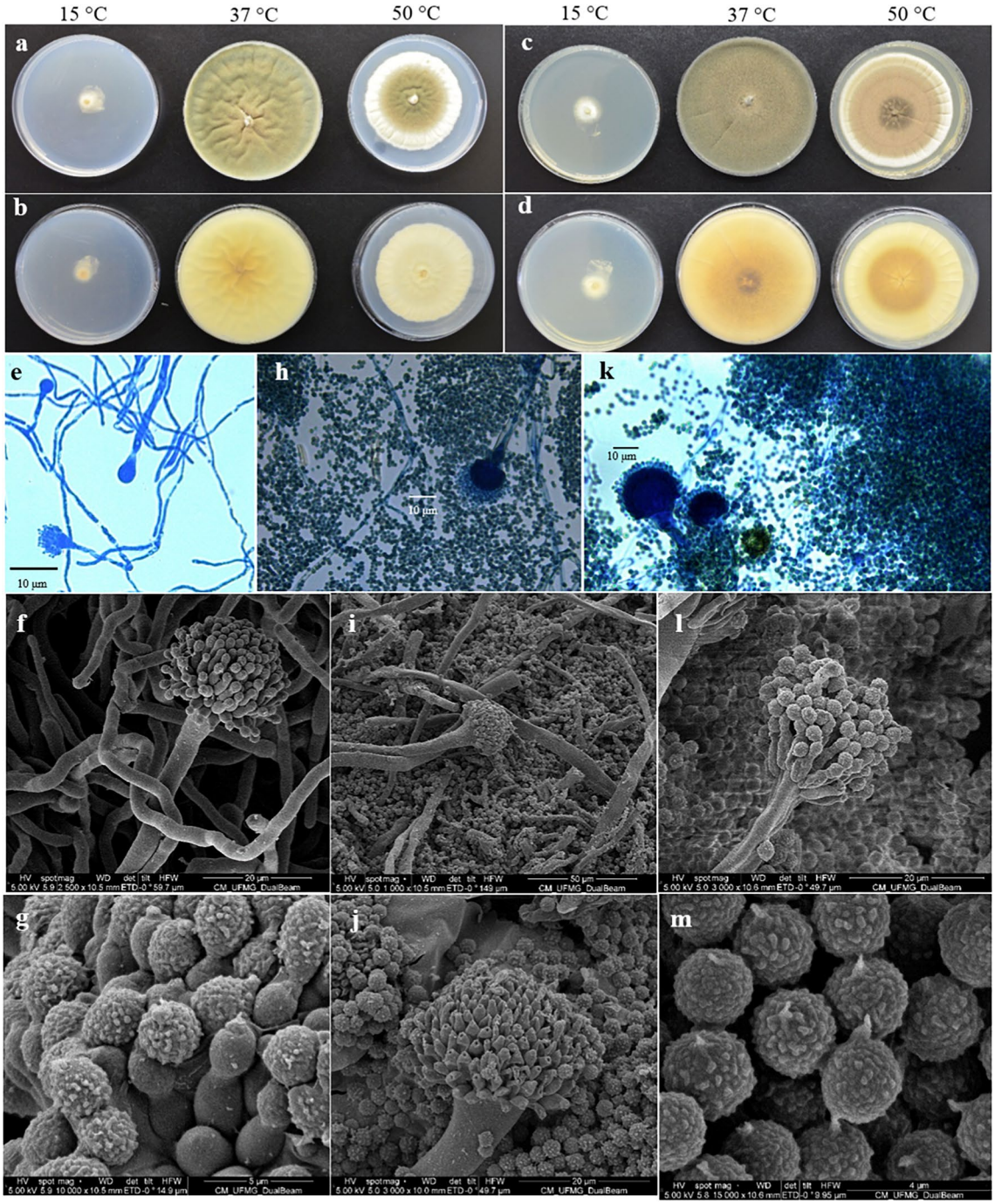

Fig. 2 Macro- and micromorphology of *Aspergillus fumigatus* UFMGCB 11829 under different temperature conditions at 7 days of growth. Fungal colonies (**a**) front and (**b**) reverse in CZ and (**c**) front and (**d**) reverse in YM. Micromorphology of the conidiophore and conidia (**e**) optical, **f** and **g** MEV at 15 °C; **h** optical and (**i** and **j**) MEV in CZ at 37 °C; **k** optical and (**l** and **m**) MEV at 50 °C

conditions [8]. In Antarctica, viable *Aspergillus* species have been recovered from substrates such as thermophilic soils (*A. calidoustus* [24]), ornithogenic soils (*A. fumigatus* [8]), penguin excreta (*A. fumigatus* [25]), and permafrost (*A. thermomutatus* [11]). The latter went on to display active growth at temperatures of 37 ºC and above. *Aspergillus fumigatus* was also obtained in 10.5% of samples collected from Antarctic penguins and pinnipeds, displaying growth capability at 15º, 28º and 37 °C [26]. Bhabhra and Askew [27] reported that *A. fumigatus* can tolerate temperatures up to 60 °C, approximately the upper temperature limit for eukaryotic organisms, and considered it a thermophilic fungus,

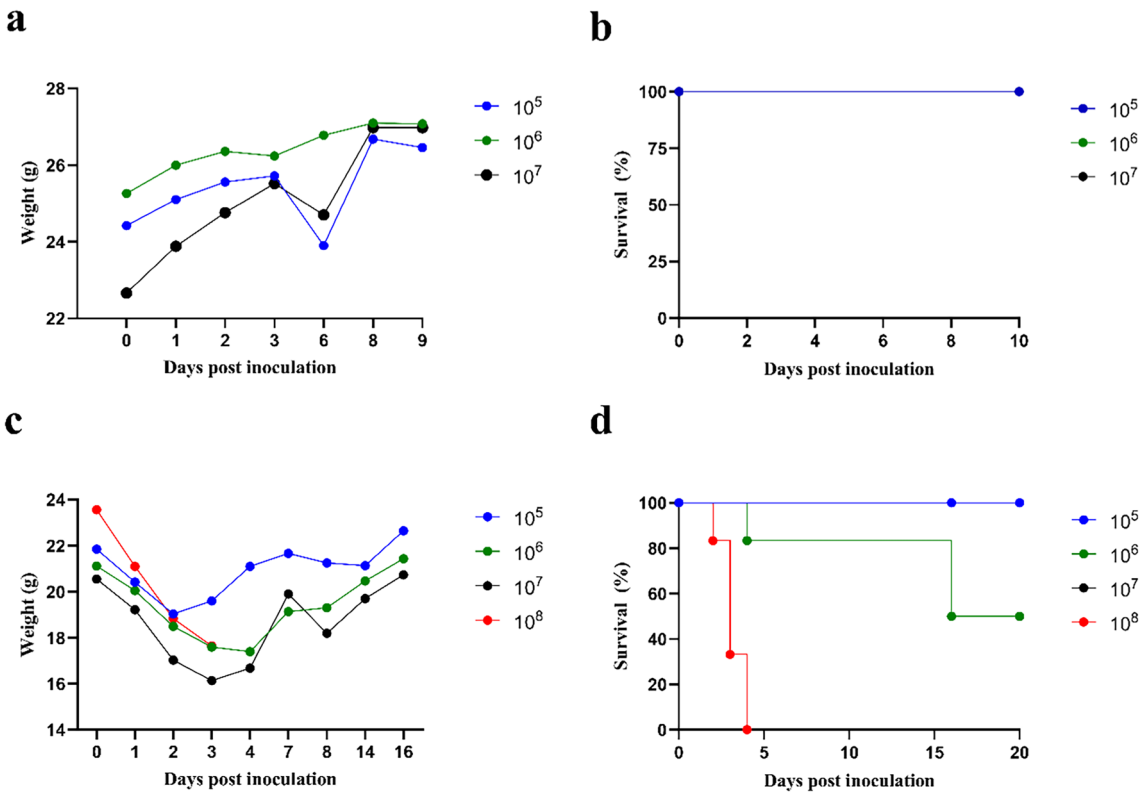

Fig. 3 In vivo assay using the Antarctic strain of *Aspergillus fumigatus* UFMGCB 11829 against healthy (**a**) weight and (**b**) survival, and immunocompromised (**c**) weight and (**d**) survival in BALB/c male mice

an important capability in the context of the pathogenesis of aspergillosis in humans. Our taxonomic study using a molecular phylogenetic approach further supports that the strain *A. fumigatus* UFMGCB 11829 is a representative of *A. fumigatus* and is closely related to multiple strains of that species that have been recognized as opportunistic pathogens of animals, including birds and immunocompromised humans [21].

In vivo effects of infection with *Aspergillus fumigatus* UFMGCB 11829 in healthy and immunocompromised BALB C mice

Aspergillus species can cause a wide spectrum of syndromes in humans, depending on the underlying immune status of the host, resulting in non-invasive forms, infection or chronic pulmonary aspergillosis, that can range from development of a fungus ball (aspergilloma) to a chronic inflammatory and fibrotic process [28]. Among the opportunistic *Aspergillus*, *A. fumigatus* is the most important pathogenic species for birds, including captive penguins [2], and immunocompromised patients, mainly through being able to cause allergic bronchopulmonary aspergillosis in asthmatic patients [3, 4, 29].

Infections in humans caused by *A. fumigatus* occur mainly through inhalation of airborne conidia, which are present in indoor and outdoor environments at concentrations typically ranging between 1 and 100 conidia m^{-3} but which can reach up to 10^8 conidia m^{-3} in certain environments [28]. Once conidia enter the bronchioles or alveolar spaces, their thermotolerance allows germination to occur and the development of mycelia [1]. During the human infection process, *A. fumigatus* expresses various resistance mechanisms that allow it to survive stress-related changes in temperature, pH, water balance, oxidative damage and antifungal host molecules [28]. Additionally, when in the lungs, *A. fumigatus* can escape from host immune system macrophages using mechanisms such as conidial resistance, melanin production and antioxidant activity [1].

Our data suggest that the presence of *A. fumigatus* UFMGCB 11829 in penguin colony soils may indicate a risk of infection of marine birds and mammals in this environment. The strain has capacity to grow and sporulate at 15° C (within, if at the upper end of the typical maritime Antarctic soil temperature range experienced in the austral summer [30]) and 37 °C (body temperature typical of different birds and mammals). However, it is also notable that the fungus only affected immunosuppressed mice in experimental exposure to different spore abundances (100% lethality with inoculum

of 10^8 conidia), which suggests it may only represent a serious risk for immunosuppressed animals (including humans).

Conclusions

Our detailed taxonomic study confirmed that the strain *A. fumigatus* UFMGCB 11829, originally recovered from ornithogenically-influenced soil obtained from gentoo penguin nests, is a representative of this opportunistic pathogenic fungal species reported in Antarctica for the first time. The strain displayed wide thermal plasticity and was capable of growth and conidia production at 15º, 37º and 50 ºC, suggesting a risk of infection in penguin colonies under natural environmental conditions, mainly in maritime Antarctica region in the summer season. In vivo pathogenicity tests using BALB C mice indicated that healthy animals did not suffer any detectable negative effects from infection with the fungus; however, that immunocompromised animals were affected by the fungus, leading to significant weight loss and death, especially at the highest spore inoculum tested (10^8). Our results also reinforce previous studies that have highlighted the presence of opportunistic pathogenic fungi in the maritime Antarctic, and of their potential risk of transfer both within and beyond the Antarctic, itself potentially exacerbated by global climatic changes.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42770-024-01326-w.

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Author contributions LHR and VNG conceived the study. LHR collected the samples. VNG cultured and identified the fungus. VNG, SSA, MCC and DAS performed the in vivo experiments. PC analysed the results and revised the manuscript. All authors read and approved the final manuscript.

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Data availability All data generated or analyzed during this study are included in this publish article.

Declarations

Competing interest The authors have no competing interest to declare that are relevant to the content of this article.

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