ORIGINAL RESEARCH

Invasive *Aspergillus terreus* morphological transitions and immunoadaptations mediating antifungal resistance

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Background and aims: *Aspergillus terreus* Thom is a pathogen of public health and agricultural importance for its seamless abilities to expand its ecological niche. The aim of this study was holistically to investigate *A. terreus* morphological and immunoadaptations and their implication in antifungal resistance and proliferation during infection.

Materials and methods: In-depth unstructured mining of relevant peer-reviewed literature was performed for *A. terreus* morphological, immune, resistance, and genetic diversity based on the sequenced calmodulin-like gene.

Results: Accessory conidia and phialidic conidia produced by *A. terreus* confer discrete antifungal resistance that ensures survivability during therapies. Interestingly, by producing unique metabolites such as Asp–melanin and terretonin, *A. terreus* is capable of hijacking macrophages and scavenging iron, respectively. As such, *A. terreus* has established a rare mechanism to mitigate phagocytosis and swing the interaction dynamics in favor of its proliferation and survival in hosts.

Conclusion: It is further unraveled that besides *A. terreus* genetic diversity, morphological, biochemical, and immunologic adaptations associated with conidia germination and discharge of chemical signals during infection enable masking of the host defense as an integral part of its strategy to survive and rapidly colonize hosts.

Keywords: HSP70, terrein, terretonin, Asp–melanin, virulence, stomata atropism

Introduction

Aspergillus terreus is a medically important pathogen associated with life-threatening states, ranging from cystic fibrosis, aspergillomas, spondylodiscitis, and periprosthetic hip-joint infection in immunocompetent hosts and lethal invasive disease in immunocompromised individuals.[1–4] An upsurge in the mortality rate of 74%–92% in transplant recipients suffering from invasive aspergillosis has been reported.[5,6] It is now understood that invasive aspergillosis sufferers often have higher incidence of cancer and organ transplantation,[7] an indication that *Aspergillus* infections could lead to sequential development of other diseases. Other than the harmful effect in medicine, *A. terreus* is the only source of the valuable drug lovastatin, used for reduction of serum cholesterol.[7] Regardless of modern therapies, ~30%–50% of invasive aspergillosis patients die for such reasons as late diagnosis, infection of the brain not efficiently treated with antimycotics, and drug-resistance emergence.[8,9] One possible reason could be the partial understanding of the survival mechanism of *A. terreus* conidia and intertwining with host defense. Another limitation has been how *A. terreus* is transported from the common site of infection, often the lungs, to distant sites, such as the central

nervous system, in immunocompetent patients, associated with fungal meningitis.[10–12] Given successful germination of *A. terreus* conidia is the key step to invasive aspergillosis, a recent proteomic study uncovered virulent proteins, such as Hog1 and MPKC, and high-level transcripts, such as the *DHO* and *TER* genes, associated with the transition stages that are a hallmark of conidia germination.[13]

Economically, *A. terreus* has influenced agriculture negatively and positively. Positively, *A. terreus* acts as a biocontrol agent against *Biomphalaria alexandrina* snails,[14] acts as a promoter of plant growth, and mitigates the adverse effects of stem-rot disease caused by *Sclerotium rolfsii*.[15] Contrary to the beneficial aspects in agriculture, *A. terreus* and other filamentous fungi are mainly responsible for severe loss of crops worldwide, damaging over 125 million tons of food grains annually.[16–19] Whether inside the seed, on the seed, or as a contaminant, evidence shows that *A. terreus* is part of the cohort of fungi that adversely affects seed viability, germination, emergence, and plant-growth vigor, and eventually hampers agricultural productivity.[20] The saprophytic nature of *A. terreus* and ability to colonize important new vegetables, such as sugar beet (*Beta vulgaris*), Jew's mallow (*Corchorus olitorius*), cucumber (*Cucumis sativus*), purslane (*Portulaca oleracea*), and eggplant (*Solanum melongena*),[20] is an indication that its current impact in agriculture is underestimated. The successful nature of *A. terreus* to cause diseases in plants and animals and/or jump hosts qualifies it as a cross-kingdom pathogen.

Each fungal infection starts with germination of conidia.[21] Because of the ease with which *A. terreus* colonizes novel niches, one could question whether there exist rare strategies based on morphological changes and adaptability during active invasion of hosts. Nonetheless, the current literature has scanty information on the mechanism by which *A. terreus* infects plant hosts. Capturing infection mechanisms in plants and drawing parallels with infection mechanisms of animals could provide new insights on the control of *A. terreus* in animals and plants. This ignored gap poses a serious public health problem, since on such plant hosts as food crops, *A. terreus* infects and liberates mycotoxins that are of public health concern. While plant and animal immunity are different, we draw parallels between the two systems and provide analysis of how *A. terreus* successfully thrives on hosts. This study provides a holistic analysis of the invasion strategies explored by *A. terreus* to mask host defenses, focusing on morphological adaptability and chemical arsenals. It is revealed that morphological changes associated with conidia germination and discharge of unique chemical signals, such as terrein, terretonin, Asp–melanin, overexpression of catalase, and HSP70, in *A. terreus* are integral arsenals employed in outwitting host defenses and mounting resistance to antimycotics. The study aims to unravel the morphological, immune, and susceptibility changes associated with conidia proliferation and chemical signal impact on plants and animals of public health concern.

Materials and methods
Database search, extraction, and synthesis
An unstructured review was performed on *A. terreus* infections, morphology, virulence, immunoresponse, and resistance based on available data in peer-reviewed literature (from Medline, Scopus, Google Scholar, and CINAHL) and gray literature in book chapters and unindexed sources, including local and global agencies, eg, the UN Food and Agriculture Organization and World Health Organization in the months of June–August 2017 (Table S1). Prevention and early-warning websites were searched using such keywords as "*Aspergillus* species", "*A. terreus*", "*A. terreus* – morphology*", *A. terreus* – resistance", "*A. terreus* – pathogenicity, and "*A. terreus* – virulence". Key MeSH terms used included "*A. terreus* health morphological adaptability", "*A. terreus* chemical metabolites and agriculture", and "*A. terreus* resistance to antimycotics". Abstract (or résumés) were read for relevance, then the full articles or documents reviewed.

A total of 30 studies were referred to for this study. We extracted *A. terreus* morphological and susceptibility changes to survival data as reported in various studies according to the criteria and methods used in each study. Further evaluation was based on susceptibility testing to antimycotics, conidia-germination strategies, and chemical signal impact on plants and animals of public health concern. The overarching data set and information generated were analyzed.

DNA extraction, PCR sequencing, and phylogeny
Comparing clinical *A. terreus* strains with that of plant origin could provide insights on their evolutionary pattern in different habitats and allow for prediction of the challenges that could be encountered should a strain from a plant infect an animal. For this course, the genomic DNA of virulent *A. terreus* (GenBank accession GI|JX155853) of potato origin[19] grown in nutrient broth (HiMedia, Mumbai, India) was isolated. The total genomic DNA was extracted from mycelium mats using UltraClean microbial DNA-isolation kits (Mo Bio Laboratories, Carlsbad, CA, USA) as described by the manufacturer. The quality and quantity of the DNA

were determined using 1.0% agarose-gel electrophoresis and nanodrop spectrophotometry (BioSpec-nano; Shimadzu, Kyoto, Japan), respectively.

For polymerase chain reaction (PCR), specific primers (forward 5'-ccgagtacaaggaggccttc-3', reverse 5'-ccgatagag-gtcataacgtgg-3') were designed for the *CAD* gene using reference *A. terreus* (GenBank EF669543.1) in Integrated DNA Technology (IDT) primer designer software. The PCR mixture was composed of 1 μL of 100 ng genomic DNA, 5 μL 10× high-fidelity standard PCR buffer, 0.5 μL deoxynucleotide triphosphate (dNTP) mix (10 mM), 1.25 μL MgCl$_2$ (50 mM), 0.5 μL of each primer, and 0.2 μL Phusion DNA polymerase (5 U/μL; Thermo Fisher Scientific, Waltham, MA, USA), resulting in a final volume of 25 μL. The cycle conditions used initially were 98°C for 30 seconds for denaturing, followed by 35 cycles of 98°C for 10 seconds, 56°C for 30 seconds, 72°C for 1 minute, and a final extension of 72°C for 7 minutes in a MasterCycler gradient thermal cycler (Eppendorf, Hamburg, Germany) targeting the 559 bp region. The amplicon was purified with a QIAquick PCR purification kit (Qiagen, Venlo, Netherlands) and sequenced with reverse and forward primers using a BigDye Terminator 3.1 cycle-sequencing kit (Thermo Fisher Scientific), coupled with an ABI Prism 3100 genetic analyzer sequencer following the manufacturer's instructions (Thermo Fisher Scientific). The sequenced *CAD* gene was analyzed in DNA Baser and submitted to the National Center for Biotechnology Information database as accession KC305600, based on 100% similarity with type isolates. Phylogenetic analysis was performed using a Mega 6.1 server.

Results and discussion
Significance of *A. terreus* conidia morphological adaptability during infection

A. terreus falls within the group of fungi whose conidia display unique morphological changes during infection. Inherently, *A. terreus* produces asexual conidia, also called phialidic conidia (Figure 1), at the tips of broom-like conidiophores.[19,22,23] In addition to phialidic conidia (PC; 1.5–2 μm diameter), *A. terreus* produces globular accessory conidia (AC; 3–6 μm diameter) emanating from colonizing hyphae.[19,22] Important factors affecting *A. terreus* virulence are inoculum size, host characteristics,[24] and the expression of terrelysin (a hemolytic protein), CgrA, CREB, PepP, MyoA, Mep, Hog1, and MPKC, secreted during germination of *A. terreus* conidia.[13] Remarkably, the rate of *A. terreus* infection increases proportionally with the pathogen inoculum, but inoculum size does not affect intrinsic host characteristics.[24,25] Therefore, it is important to understand key morphological aspects of PC and AC, and how they influence host colonization.

High-resolution staining has revealed that AC have a uniform ring-like pattern of the β-glucan layer compared to PC, whereby a nonuniform contour of the β-glucan layer was observed[26] in accordance with a previous thesis that AC and PC could have different cell-wall composition.[23] Equally, AC are multinucleated and undergo multipolarization, which leads to several hyphae capable of inducing heightened inflammatory responses in a pulmonary model of aspergillosis.[25] Based on the uniqueness of AC and PC, it has been suggested that there exist differential immunoresponses to distinct conidia types in an animal model,[27] opening other avenues for investigation.

A. terreus produces different invasive structures on plant host

As most studies on *A. terreus* have focused on animal models, it remains unknown whether PC and AC exhibit similar germination strategies to invade all hosts. More recently, phytopathology data revealed that PC also undergo multipolar germination (Figure 1), marked by three to four delicate hyphae, to invade potato-leaf tissue.[28] Interestingly, it was noted that by 8 hours after germination of PC, hyphae had differentiated into pigmented irregular protuberances (0.2–0.5 μm in diameter) and failed to penetrate epidermal cells and stomata[28] thus exhibiting stomata atropism (ie, inability to penetrate via the stomata). Although the role of irregular protuberances in infection is unknown, the authors indicated that irregular protuberances form recursive hubs for efficient colonization of potato leaf. While the infection process in plants and animals are different, commonalities lie in the ability of *A. terreus* to produce PC and AC, which are potent virulence factors.

Spatial distribution pattern of AC on colonizing hyphae and host sensing

Little investigation has been performed on morphological changes in PC and AC in host sensing during germination and infection. Two patterns of distribution of AC on colonizing hyphae have been identified. Based on animal models, AC arise on the surface of colonizing hyphae in an alternating thorn-like pattern[23,26] and irregularly spaced. Interestingly, it has been found serendipitously that AC can arise in an opposing pattern perpendicularly to one another and irregularly spaced on colonizing hyphae during infection
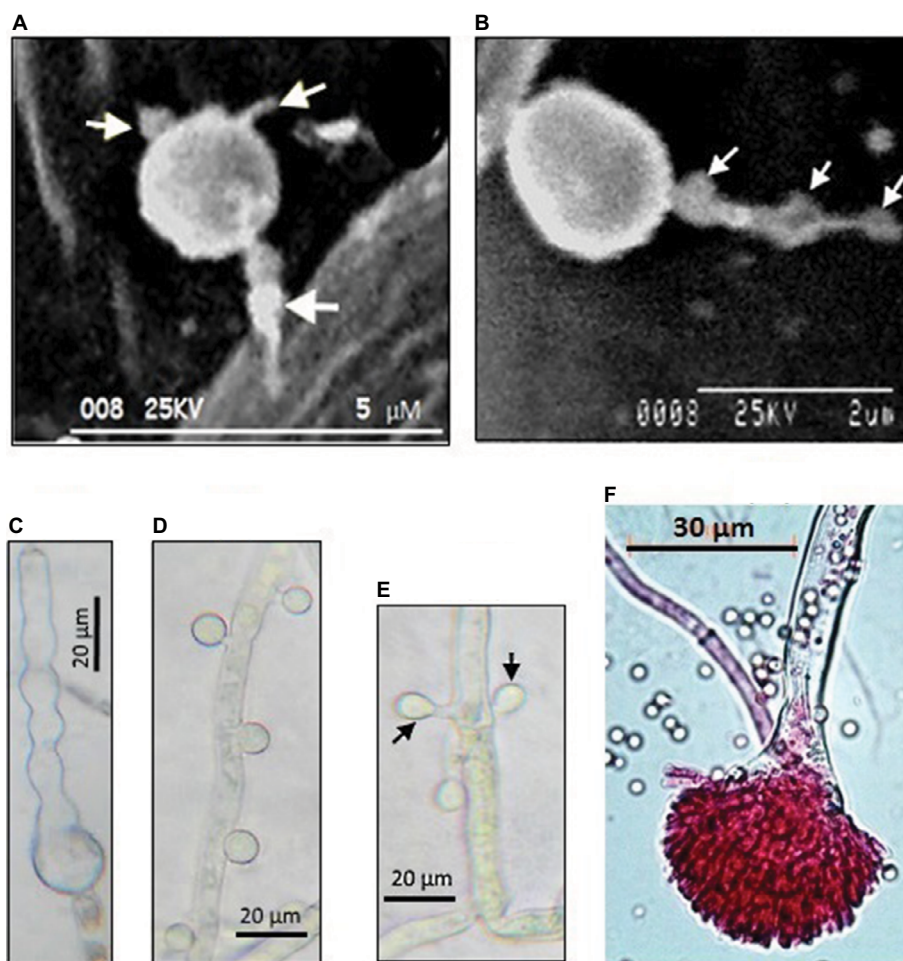
Figure 1 Morphological differentiation of *Aspergillus terreus* (GenBank accession KC305600) conidia during infection of potato leaf.
Notes: (**A**) A germinating phialidic conidium infecting abaxial leaf of potato produces multipolar hyphae (tagged with arrows); (**B**) hyphae from phialidic conidium could produce irregular protuberance (tagged with arrows); (**C**) irregular protuberance is pigmented, probably melanin, which plays a protective role; (**D**) thorn-like pattern of accessory conidia; (**E**) opposing accessory conidia; (**F**) conidiophores stained with rose Bengal as previously described by Louis et al.[19] Images reproduced from Louis B, Sayanika DW, Pranab R, et al. Invasion of *Solanum tuberosum* L. by *Aspergillus terreus*: a microscopic and proteomics insight on pathogenicity. *BMC Res Notes*. 2014;7:350.[28]

of potato-leaf epidermis, but not on potato slices in vitro[28] (Figure 1). The authors suggested that a specific host-tissue signal could be responsible for the production and alignment of AC on infection hyphae. With comparison of patterns of AC development on colonizing hyphae in animal[26] and plant[28] models, it is not unreasonable to hypothesize that AC could be playing a sensing role that aids in recognizing a suitable host during infection.

To gain insight into the role of AC in host sensing, it could be interesting to profile the whole proteome of AC and predict glycosylphosphatidylinositol-anchored proteins, which often act as effectors and participate in cell recognition, and compare it with those of PC, firstly to understand the amplitude of PC in inflammatory response in animals. Secondly, such investigation could provide novel insights into how AC and PC subdue the host during infection. The proposed proteomic approach could help shed light into why

A. terreus AC elicit a superior immunoresponse, as observed via ex vivo alveolar macrophages and in vivo intratracheally challenged mice.[26] It has been found that clinical isolates of *A. terreus* produce morphologically diverse AC and relatively similar AC morphologies at the strain level,[23] which could be a function of genetic diversity. Recently, it was shown that isolates of *A. terreus* specifically from dried grape showed significant genomic diversity based on random amplification of polymorphic DNA–PCR data, although morphological differences were not significant.[29]

Using the sequenced *CAD* gene from *A. terreus* (Gen-Bank accession KC305600; 559 bp), which causes foliar potato blight, and by comparing the sequence with those of clinical strains, we found evidence of divergent evolution due to point mutations (Figure 2). Morphological features based on conidia, conidiophores, and universal rDNA help in identification of *A. terreus*, but cannot effectively
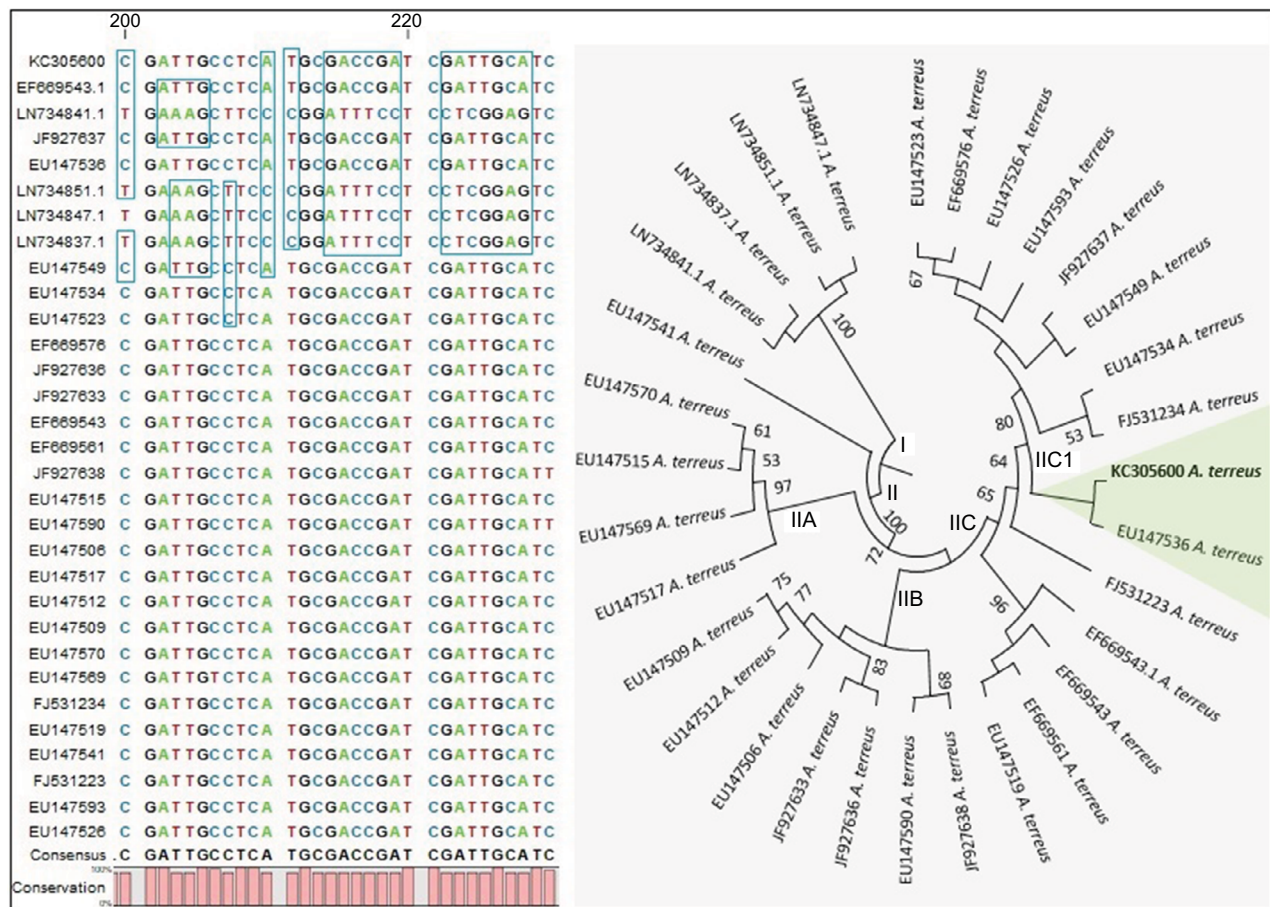
Figure 2 Molecular phylogenetic analysis by maximum likelihood (ML) based on the K2 substitution model[55] using calmodulin gene.
Notes: Evolutionary analyses were conducted in MEGA6.[56] Regions of significant point mutations in the shown sequence alignment are highlighted, and main clades I and II are shown. The ML tree indicated that clade II containing *Aspergillus terreus* KC305600 encompasses highly diverse strains, represented by subclusters IIA, IIB, IIC, and IIC1. The highlighted taxa showed a close relationship between a clinical strain (ie, EU147536) and strain KC305600, which causes foliar blight of potato.

discriminate isolates of *A. terreus*, because of overlapping morphological traits and genome plasticity at rDNA region.[28,37] We used the *CAD* locus, because it has been shown to discriminate different isolates of *A. terreus* accurately.[37] From the 31 sequences computed, 188 patterns were found from a total of 763 sites, and 313 sites were without polymorphisms (41.02%). The nucleotide frequencies were A 22.73%, T/U 25.17%, C 28.07%, and G 24.03% at a discrete γ-distribution of 23.5. The best substitution-model parameters were determined based on the Akaike information criterion, corrected (1,642.868) and Bayesian information criterion (3,864.291). The generated phylogenetic tree had the highest log likelihood: −1,640.72. The estimated average evolutionary divergence overall in *A. terreus* strains in the data set was 1.47±1.1. Based on the high-level clustering pattern in clade II, and considering that only one clinical strain clustered with KC305600 indicated that *A. terreus* strains were highly diverse, this diversity was able to be translated at the morphological level during host colonization (Figure 1). This genetic variability could explain in part the differences in the spatial distribution pattern of opposing AC on colonizing hyphae of *A. terreus* KC305600, not yet observed in clinical isolates (Figure 1). Therefore, comparative proteomics could delineate whether the different morphological forms of AC bear similar effector proteins during infection.

Impact of *A. terreus* use of chemical signals to mask infectious activity

A. terreus produces terretonin, a meroterpenoid that acts as an iron scavenger,[30] thus affecting vital physiological processes in the hosts. Often, pathogenic fungi receive morphological and chemical signals from plant cells that are direct consequences of fungal invasion,[31] but this may not be true for animal cells. Nonetheless, *A. terreus* is known to produce a vast number of metabolites.[32] Because chemical signals are often metabolites used for defense against predators and

competitors, for chemical communication, and to manipulate host cells,[28] *A. terreus* is consequently a formidable pathogen. Importantly, *A. terreus* produces terrein, a metabolite expressed under the influence of environmental stimuli[33–35] that confers an added advantage for invading diverse hosts. For example, in mammals, terrein reduces tyrosinase protein levels and induces a decrease in melanin synthesis via the extracellular signal-regulated protein-kinase pathway and microphthalmia-associated transcription factor.[36] A previous study elucidated that terrein inhibited the growth of bacteria, fungi, and mammalians cells,[34] hence signifying that *A. terreus* senses the host or immediate environment. Irrespective of the inflammatory potential of AC, terrein also acts as an antioxidant and anti-inflammatory agent,[33] indicating that a given *A. terreus* strain could infect animals yet mask inflammation, regardless of the presence of AC on colonizing hyphae.

Overproduction of AC and PC during infection is an effective survival strategy

Aspergillus conidia display high tolerance to heat (~85°C),[37] germinate at low water activity (<0.6), show high tenacity for pressure and chaotropic agents, and can survive for over 60 years.[38] While strong superficial colonization of potato leaves is marked by abundant production of PC and AC,[22] the scenario is different in invasive aspergillosis. For instance, in severe invasive aspergillosis characterized by angioinvasion, colonizing hyphae can spread hematogenously and equally penetrate deep organs.[12,39] It is evident such a colonization process encounters resistance and immunoresponses that can alter the survival of conidia or affect their production. One important aspect to consider is how strong and weak host responses could influence survival of *A. terreus* vis-à-vis PC and AC. Hypothetically, when one considers that strong host response leads to cellular restriction of infection, then there is a high chance of abundant production of PC. This is because PC is the principal form of survival in adverse conditions, and such a scenario could lead to subsequent fatal invasive aspergillosis in those associated with long-term persistence of PC and liver degeneration.[33] On the other hand, if host response is weak at the onset of colonization, PC germinates and produces colonizing hyphae that bear AC, which enhance virulence and severity of the infection. This intertwining in the developmental stages associated with infection that gives rise to PC and AC (Figure 3) offers a highly successful means of survival under adverse conditions.

Using vacuum ultraviolet irradiation (100–180 nm), low temperature, and a combination thereof to evaluate *A. terreus*

conidia survival under simulated interplanetary conditions led to the deduction that interplanetary survival of conidia depends mainly on dehydration stress in vacuum.[40] Nonetheless, PC-survival probability extended to 4 days in vacuum (~34%), due to exudation of proteins on the external surface membrane, which prevents excessive conidia dehydration.[40] Results from this interplanetary simulative study provided clues that only drastic sterilization procedures would eliminate *A. terreus* under clinical settings.

Mitigating antimycotic effect through overexpressed *A. terreus* catalase and HSP

Research has shown that *A. terreus* inherently displays resistance to frontline polyene–amphotericin B, and in some cases 98% of clinical isolates showed resistance.[25] The *A. terreus*-resistance mechanism to amphotericin B is not clearly understood, but it has been suggested that overproduction of catalase may contribute to amphotericin B resistance.[41] *Aspergillus* spp. have the ability to overexpress catalase and hyperoxide dismutase during infection and lower intracellular reactive oxygen species, thus increasing amphotericin B resistance.[42,43] It worth mentioning that colonization of potato tubers is marked by differential expression of catalase–peroxidase complexes, often involved in signal-transduction pathways and cross talk in pathogenic responses.[28] Another line of evidence indicates that the main cause of amphotericin B resistance in *A. terreus* is cytosolic HSPs, which naturally play a protective role against various stress responses (Figure 3). Experimentally, an HSP70 inhibitor (eg, pifithrin-μ) considerably enhances the susceptibility of resistant *A. terreus* strains to amphotericin B, but nonetheless has little impact on susceptible strains.[44] By considering these resistance mechanisms,[41,43,44] it is suggested that *A. terreus* may switch between catalase overexpression and HSP chaperones to mount strong resistance to amphotericin B in immunocompromised patients under therapy. It could be possible that *A. terreus* uses diverse chaperones that nullify antifungal effects as part of its strategies to counter and survive adverse conditions during infection.

Assessing AC ability to confer higher resistance to *A. terreus* than PC

At this point, one could be tempted to determine whether AC and PC exhibit equal level of resistance against elite antifungal drugs to ensure survival during infection. To investigate amphotericin B and azole (eg, voriconazole) resistance further in *A. terreus*, Lass-Flörl et al[44] used PC and AC from 31 strains and determined the minimum inhibitory concentration
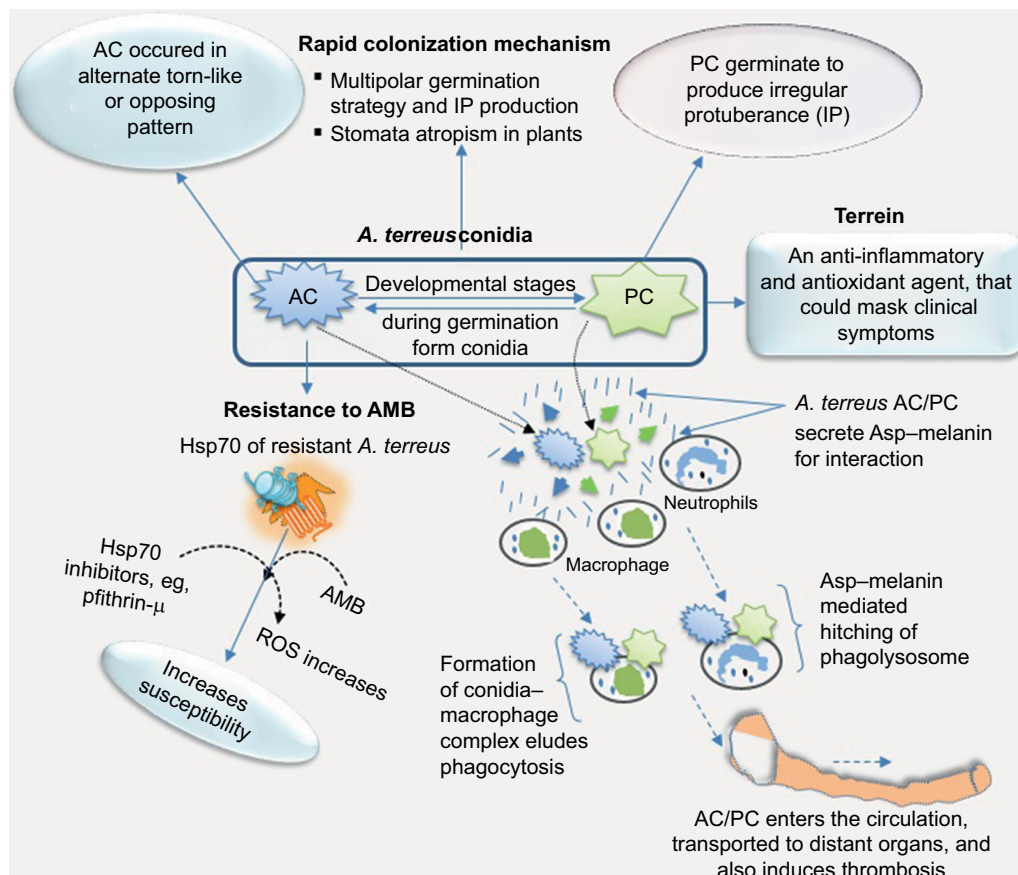
Figure 3 *Aspergillus terreus* during infection of putative host adopts a formidable means of survival.

Notes: *A. terreus* exploits its cytoplasmic chaperones, such as HSPs, as one of its counterresponses to adapt and mount resistance to amphotericin B (AMB), and this counterresponse could be associated with upsurge in reactive oxygen species (ROS). The interconnection between phialidic conidia (PC) and accessory conidia (AC) developmental stages on infection hyphae allows the pathogen to adapt to homeostatic changes and other adverse conditions in hosts. Production of Asp–melanin, which hijacks innate immunity, indicates that *A. terreus* could colonize all organs of the animal body. On the other hand, in plant host, stomata atropism (ie, inability to colonize hyphae to penetrate epidermal cells via the stomata pore) allows *A. terreus* to colonize superficially at a faster rate as a necrotroph while secreting metabolites that trigger necrosis and mitigate the host defense system.

(MIC) of amphotericin B and voriconazole vis-à-vis the strains. It was found that 90% of the 31 strains had an MIC of 2.5 µg/mL of amphotericin B as it relates to PC, but 5 µg/mL amphotericin B for AC was needed.[45] It was also found that an MIC of 1 µg/mL of voriconazole was required for PC, while the MIC of voriconazole for AC stood at 2 µg/mL.[45] Even though the relationships among amphotericin B, PC, and AC were not statistically significant, striking disparities in MIC were slightly higher than for hyphae, thus suggesting that AC and PC confer discrete antifungal resistance to *A. terreus* that could ensure survivability during treatment. Furthermore, since invasive aspergillosis leads to tissue necrosis at points of infection and reduces leukocyte penetration, as well as the effectiveness of antimycotics,[39] all these attributes enhance *A. terreus* resistance. Importantly, AC have a unique surface architecture (smooth, thick, and apparently unpigmented cell surface), cell-wall composition (less cell membrane ergosterol), and are multinucleated (three to seven

nuclei) in contrast to PC,[23,26] and that could be an underlying cause of dissimilarities in MIC values. Intriguingly, cases of *A. terreus* resistance continue to rise, and may be intrinsic, acquired from exposure to azole fungicides or antifungal drug exposure, or in antifungal drug-naïve patients.[46] Therefore, treatment of invasive aspergillosis could require personalized drugs. The rationale behind this is that most antifungal drugs are primarily designed for fungicidal activity, and consequently it is not clear whether commercial antifungal drugs target AC or PC more. Firstly, antifungal drugs that primarily target PC more could be of great medical importance, because only PC are airborne, while AC are formed only as accessories on infection hyphae during colonization. Secondly, it could allow for deducing best-treatment regimens based on combination therapy. Thirdly, this investigation could lead to repurposing of some antifungal drugs to effectively counter antifungal drug resistance. Repurposing and personalizing antifungal drugs could shed light on the existing concept that

antifungal-combination therapy offers no definite advantage over montherapy.[47–49]

Understanding of systemic colonization of *A. terreus* production of pigmented and acidic conidia

While most fungi associated with lung disease remain localized in the disease area, invasive aspergillosis caused by *A. terreus* has been detected in the central nervous system,[12] challenging minds as to the means of transport to distant organs. It has been found that *A. terreus* produces pigmented PC that partially protects it from predators, such as macrophages. Conceptually, innate immunity (ie, neutrophils and macrophages) engulfs and eliminates harmful microbes. Conidia of some *Aspergillus* spp. produce certain types of dihydroxynaphthalene (DHN)–melanin, which inhibits phagolysosome acidification.[50] *A. terreus*, on the other hand, lacks genes for the synthesis of DHN–melanin, but produces a different kind of pigment called Asp–melanin, which partially favors acidification.[51] Interestingly, Asp–melanin plays a key role in *A. terreus* survival by conferring resistance against ultraviolet light, as well as hampers phagocytosis by soil amoeba.[50] Remarkably, the failure of Asp–melanin to inhibit acidification of phagolysosomes suggests it contributes to the survival of *A. terreus* conidia in acidic environments.[51] Most fungi conidia are usually neutral pH-loving, contrary to conidia of *A. terreus*, which are acid-loving. It has been suggested that the acid-loving property allows conidia of *A. terreus* to survive distant transport by hitching on immune cells as transport vehicles throughout the body.[51] By hijacking the host macrophages (Figure 3), *A. terreus* has established a rare strategy to mitigate phagocytosis, and readily induces thrombosis and swings interaction dynamics in favor of its proliferation.

Outlook on *A. terreus* pathogenicity and invasiveness

Morphological changes during germination in cross-kingdom fungi during infection are an intrinsic strategy to colonize hosts rapidly, and improve their fitness on new hosts. *A. terreus* and *A. fumigatus* are the leading causes of hard-to-treat invasive aspergillosis. Recent studies have aimed to identify pathogenicity factors in *Aspergillus* spp. For instance, it was shown that *A. fumigatus* produces galactosaminogalactan (Gal), which is immunosuppressive, induces apoptotic death of neutrophils, and favors *A. fumigatus* infection.[53] Also, hydrophobin a virulence factor on spores of *Aspergillus* spp., masks dectin 1/2 recognition of conidia, impairs neutrophil

recruitment, increases fungal survival,[52,53] and hence prevents immunoresponse. More recently, conidial melanin, hydrophobin, and Gal were revealed to be important pathogenicity factors that modulate platelet activity and impact immunoresponse, inflammation, and thrombosis in infected patients.[54] It could be interesting to evaluate the effect of terrein on melanin, Gal, and hydrophobin mutants of *A. terreus* to gain insights into the extent to which terrein overproduction might mask PC and AC detection by immune cells. Finally, it will be fascinating to identify stimuli that trigger differentiation of colonizing hyphae into AC and irregular protuberance, and investigate whether terrein can inhibit or stimulate the production of AC during infection.

Conclusion

We found that *A. terreus* adopts diverse strategies to cause invasive aspergillosis, which results in morbidity and mortality in patients with hematological malignancies and blight diseases in plant hosts. The strategies identified include divergent evolution between isolates and adoption of different morphological forms during infection. Importantly, the interlocking relationship between AC, PC, and delicate colonizing hyphae confers a discrete level of resistance to antifungal drugs. Remarkably, the potential of *A. terreus* conidia to produce chemical signals that mask infectious activity enable the fungus to be highly successful.

Acknowledgments

Disclosure

The authors report no conflicts of interest in this work.

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Supplementary material

Table S1 An unstructured review performed on *Aspergillus terreus* infections, morphology, virulence, immune response, resistance, and combination-therapy in databases up to August 2017

Reference	Key message
Vincken et al[1]	Immunoactivation: aspergillosis associated with elevated IgE above (1,000 IU/mL) in patients aged >43 years
Dunne et al[2]	Resistance to azoles antifungals: despite good in vitro susceptibility to azoles, *A. terreus* from a 10-year-old was not eradicated during therapy; of note, sera levels of voriconazole, itraconazole, and posaconazole were 0.2–1.4 mg/L, 0.34–1.44 mg/L, and 0.41–0.4 mg/L, respectively (reference ranges 42 mg/L, 40.5 mg/L, and 5–15 mg/L for voriconazole, itraconazole, and posaconazole, respectively)
Comacle et al[3]	Combination therapy against *A. terreus* infection in a 20-year-old: 400 mg twice-daily dose of voriconazole, followed by suspension, and then introduction of caspofungin on day 17 at a loading dose of 70 mg IV, followed by 50 mg IV once daily for 40 days
Bartash et al[4]	Combination therapy against *A. terreus* infection in a 54-year-old: used voriconazole-impregnated cement and many weeks of oral delayed-release posaconazole to maximize treatment
Paterson and Singh[5]	Degree of invasiveness of *A. terreus*: *A. terreus* accounted overall for 3% of *Aspergillus* infections and 20% of the invasive mold infections in hematopoietic stem-cell-transplant recipients were caused by *A. terreus*
Taccone et al[6]	Epidemiology of invasive aspergillosis: global occurrence of infections and mortality
Elsawy et al[7]	Aspergillosis of the central nervous system: first isolation of *A. terreus* in cerebrospinal fluid and combination therapy of liposomal amphotericin B and voriconazole could not save the 16-year-old immunocompetent patient
Thakur and Shankar[8]	Virulence elements: proteomic study that uncovered virulent proteins, such as Hog1 and MPKC, and high-level transcripts, such as *DHO* and *TER* genes associated with the transition stages that are a hallmark of conidia germination
Saad et al[9]	Biocontrol agent: as a biocontrol agent, *A. terreus* kills *B. alexandrina* snails at sublethal concentration (LC_{50}) 1.05%
Kozakiewicz[10]	Pathogenesis of cereals
Louis et al[11]	Virulence on potato plants
Abdelwehab and El-Nagerabi[12]	Pathogenesis of cereals
Balajee[13]	Morphology: morphological in-depth analysis of conidia and conidiophores of *A. terreus*
Deak et al[14]	Morphology: morphological insights on the conidia of *A. terreus* cell wall during germination
Lass-Flörl[15]	Virulence: comparative analysis uncovering how the amount of inoculum and host intrinsic nature influences *A. terreus* colonization
Slesiona et al[16]	Invasive aspergillosis: development of three infection models for long-term persistence of *A. terreus* conidia; *A. terreus* produces secondary metabolites that have hepatotoxic capacity
Deak et al[17]	Pathogenicity and virulence: mouse alveolar macrophages showed stronger inflammatory cytokine/chemokine response when challenged with accessory conidia (AC) than with phialidic conidia (PC) in both ex vivo and in vivo experiments, correlating with greater exposure of β-glucan exhibited by AC; work also demonstrated that AC is multinucleated
Vyas[18]	Immunoresponse: magnitude of immunoresponse to *A. terreus* infection depends on the type of conidia
Louis et al[19]	Pathogenicity and morphological transitions: associated proteomics with microscopy to decipher the colonization process of potato leaf and tuber *by A. terreus*
Haas[20]	Virulence and metabolite: secretion of terrein metabolites in active growth phase of *A. terreus*
Zaehle et al[21]	Virulence and metabolite: secretion of terrein metabolites in active growth phase of *A. terreus*
Park et al[22]	Virulence and metabolite: secretion of terrein metabolites in active growth phase of *A. terreus*
Sarantopoulou et al[23]	Resistance to extreme conditions: use of extreme solar irradiation in vacuum ultraviolet (110–180 nm) spectral region; *A. terreus* high survival probability after 4 days in vacuum (~34%) because of exudation of proteins on the external membrane, thus preventing further dehydration of spores
Blum et al[24]	Antifungal resistance: high-level production of catalase in *A. terreus* may contribute to AMB resistance, since oxidative damage is implicated in AMB action
Moore et al[25]	Antifungal resistance
Takasuka et al[26]	Antifungal resistance
Lass-Flörlet al[27]	Antifungal resistance: AC and PC confer discrete resistance to *A. terreus*
Sutton et al[28]	Antifungal resistance
Geib et al[29]	Immunohijacking mechanism: *A. terreus* produces Asp–melanin, confers resistance against ultraviolet light, and hampers phagocytosis by soil amoeba; remarkably, Asp–melanin did not inhibit acidification of phagolysosomes, thus likely contributing specifically to survival of *A. terreus* conidia in acidic environments

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