Asian Journal of Mycology 4(1): 1–9 (2021) ISSN 2651-1339

www.asianjournalofmycology.org **Article**

Doi 10.5943/ajom/4/1/1

Unusual thermal resistance of spores of mesophilic fungi

Rajamani T[1], Govinda Rajulu MB[1], Murali TS[2] and Suryanarayanan TS[1*]

[1]Vivekananda Institute of Tropical Mycology, Ramakrishna Mission Vidyapith, Chennai, India
[2]Department of Biotechnology, School of Life Sciences, Manipal Academy of Higher Education, Manipal, India

Rajamani T, Govinda Rajulu MB, Murali TS, Suryanarayanan TS 2021 – Unusual thermal resistance of spores of mesophilic fungi. Asian Journal of Mycology 4(1), 1–9, Doi 10.5943/ajom/4/1/1

Abstract

Thermophilic fungi are well-known for their ability to grow at high temperatures of 50–60ºC. However, our previous research demonstrated the exceptional thermal resistant capability of the spores of some mesophilic fungi. These fungi, isolated from leaf litters of forests that experience frequent ground fires, were resistant to dry heat at 100–115ºC. To determine whether these high thermal resistant spores are unique to fungi in flammable forests, we screened leaf endophytes of trees of a mangrove forest that rarely experience ground fire due to its inundated forest floor. Of the 129 endophyte isolates tested, the spores (conidia/ascospores) of 27 and 13 isolates survived an exposure to dry heat at 100ºC for 2 hrs and 4 hrs respectively. The ascospores of *Chaetomium globosum* isolated from the leaves of *Rhizophora stylosa* survived an exposure to 100ºC for 10 h. Presence of melanin synthesis inhibitor reduced the thermal resistance in this fungus suggesting that melanin plays a role in the thermal protection.

Key words – Ascospore – Conidia – Heat resistance – Melanin

Introduction

Generally, fungi are mesophilic having temperature optima between 25–35ºC for their growth (Cooney & Emerson 1964). A few species are able to grow at temperatures above 50ºC and are termed thermophilic fungi (Maheshwari et al. 2000). Although mesophilic fungi cannot grow at temperatures beyond 35ºC, Suryanarayanan et al. (2011) reported that the spores of some mesophilic fungi from dry tropical forests which experience episodic forest fires survive exposure to 100–115ºC for 2 h. Although the growing mycelia of these fungi are not heat-tolerant, this work reports that their spores represent the most heat-resistant eukaryote cells on record. The authors attributed this thermal resistance of spores to possible adaptations of these fungi to survive fires. To determine if such high thermal resistance is restricted to fungi of flammable habitats, we chose to study fungi from a forest which is not exposed to fire. We screened the spores of 129 isolates of foliar endophytic fungi of mangroves trees of the Andaman Islands for their heat tolerance. Mangrove trees here do not experience forest ground fire as they grow along the coast with permanently submerged roots.

The dark brown melanin pigments of fungi though may not be necessary for their growth and development, play an important role in their survival by protecting them against abiotic stressors including UV irradiation, desiccation, and temperature extremes (Bell & Wheeler 1986). Hence, we studied the probable role of melanin in the thermal resistance of spores of one of the fungi, which

Submitted 16 October 2020, Accepted 23 December 2020, Published 6 January 2021
Corresponding Author: T.S. Suryanarayanan – e-mail – t_sury2002@yahoo.com

showed extraordinary heat tolerance.

Materials & Methods

We screened fungal endophytes isolated in our earlier study (Rajamani et al. 2018) from the leaves of 20 mangrove species growing in south Andaman Island, in the Bay of Bengal region, India.

Test for thermotolerance

The method of Suryanarayanan et al. (2011) was followed. A sterilized cover glass was used to scrape the surface of a sporulating culture in the agar plate. Such cover glasses bearing the spores (conidia or ascospores) were placed in empty sterile glass Petri dishes without the lid and incubated in a pre-heated hot air oven fitted with a digital thermometer. The oven temperature was set at 100^{o}C and regularly verified with a mercury thermometer. The cover glasses with the spores were incubated for 2 or 4 h. After incubation, the cover glasses were removed from the oven, incubated at 25^{o}C for 30 minutes, and then placed on fresh Potato Dextrose Agar medium contained in Petri dishes. Petri dishes were incubated at 25^{o}C for seven days and examined for growth. Those fungi, which tolerated a 4 hrs treatment, were tested for tolerance to heat by extending the treatment period to 6, 8 or 10 h. All experiments were in triplicates and repeated three times.

Identification of endophytes

The fungi were identified based on their microscopic characteristics (Ellis 1971, 1976, Sutton 1980, Onions et al. 1981, Ellis & Ellis 1988). Fungi whose species could not be confirmed but differed from each other were given species numbers. The *Diaporthe* species had been identified using the molecular method in our earlier study (Rajamani et al. 2018). The ascospores of one species of *Chaetomium*, which exhibited a high thermal resistance, was identified as *C. globosum* based on ITS sequence method in the present study.

Molecular identification of thermotolerant fungus

The identity of a *Chaetomium globosum* isolated from the leaves of *Rhizophora stylosa* that exhibited a high thermotolerance was established using the molecular method as follows. Fungal culture grown in PDA medium for seven days was collected and used for genomic DNA extraction (Sawmya et al. 2013). The obtained DNA was re-suspended in 50 µl of sterile MilliQ water. PCR amplification of the internal transcribed spacer region was carried out using ITS5 and ITS4 primers (White et al. 1990). The PCR conditions were as follows: 95°C for 10 minutes, 30 cycles of 95°C for 60 seconds, 55°C for 60 seconds and 72°C for 90 seconds, and finally at 72°C for 10 minutes. The PCR product was further purified and sequenced using an automated sequencer (ABI 3130 Genetic Analyzer) using ITS4 primer. After manual editing, we checked the sequence for the closest match in the NCBI database using the Blastn algorithm. The sequence was submitted to NCBI GenBank database with the accession number MT125865 and the fungus was deposited in the National Fungal Culture Collection of India (NFCCI), Agharkar Research Institute (ARI), Pune, India (accession number NFCCI 4863).

Phylogenetic analysis

Based on the Blast result, ITS sequences of 21 closely related sequences from type specimens from the NCBI database were downloaded and aligned with our sequence using ClustalW (Thompson et al. 1994) with the default settings. After manual adjustment of the alignment, an evolutionary tree was inferred using the maximum likelihood method following the Kimura 2-parameter model using MEGA version 6 (Kimura 1980, Tamura et al. 2013). A bootstrapping analysis was carried out (1000 replications) to obtain branch support values. For constructing the tree, all positions with less than 95% site coverage were removed, and the tree with the highest log-likelihood was selected. Similarly, the identity of all the *Diaporthe* isolates from this study was established in our earlier study using a molecular approach (Rajamani et al. 2018).

Test with melanin synthesis inhibitor

Tricyclazole, a melanin synthesis inhibitor, was dissolved in ethanol and added to autoclaved and cooled PDA medium to prepare a series of concentrations at 1, 10, or 100-µg ml^{-1} (Suryanarayanan et al. 2004). This medium was inoculated with a 5 mm diameter plug of mycelium cut from the margin of an actively growing colony and incubated for 20 days at 25 ± 2°C in the dark. Spores from such colonies were screened for thermotolerance.

Results

Out of the 129 endophyte isolates tested, the spores of 27 and 13 isolates survived exposure to dry heat at 100°C for 2 hrs and 4 hrs, respectively (Table 1). Ascospores of all the seven *Chaetomium* isolates tolerated 4 hrs of exposure. Of the ten *Pestalotiopsis* isolates screened, five survived 4 hrs of exposure; of the two *Curvularia* spp., the conidia of one tolerated 2 hrs treatment while that of the other could withstand 4 hrs of heating. Conidia of *Alternaria* sp.1, *Aspergillus niger*, *Cladosporium cladosporioides*, *Curvularia* spp., and *Trichoderma* spp., and ascospores of *Emericella nidulans* tolerated exposure to 2 hrs of heating. Of the 28 isolates of *Colletotrichum/Glomerella* screened, only one survived exposure to 2 hrs of heating. The conidia of none of the 18 isolates of *Diaporthe* belonging to ten species, 12 isolates of *Phyllosticta capitalensis* and ten isolates of *Nodulisporium* spp. were heat-tolerant (Table 1). The spores of 13 isolates, which survived heating for 4 hrs, were tested with longer durations of heat treatment. While nine and seven of these isolates tolerated exposure to 6 hrs and 8 hrs of heating respectively, the ascospores of *C. globosum* isolated from the leaves of *Rhizophora stylosa* survived exposure to 100°C for 10 hrs (Table 2). In the presence of melanin synthesis inhibitor at a concentration of 100 µg ml^{-1}, the colonies of *C. globosum* were reddish and its ascospores could not survive exposure to 100°C for 8 and ten hours (Table 3).

The *Chaetomium* isolate, which could grow at 100°C for 10 hrs, was identified using a molecular method. Further, a maximum likelihood tree, constructed by comparing the ITS sequences of closely related sequences obtained from type specimens available in the public database, showed that our sequence clustered with high bootstrap support with sequences belonging to *C. globosum* (Fig. 1).

Table 1 Thermotolerance of spores of mangrove leaf endophytes

Endophyte	No. of isolates screened	No. of isolates surviving exposure to 100°C	
		2 Hrs	4 Hrs
Alternaria sp.	4	1	
Aspergillus niger	3	3	
Aureobasidium pullulans	1		
Chaetomium globosum	5	5	5
Chaetomium spp.	2	2	2
Cladosporium cladosporioides	6	1	
Colletotrichum gloeosporioides	11	1	
Colletotrichum spp.	6		
Corynespora sp. 1	3		
Curvularia spp.	2	2	1
Diaporthe discoidispora	3		
Diaporthe eucalyptorum	1		
Diaporthe hongkongensis	2		
Diaporthe kyushuensis	1		
Diaporthe liquidambaris	1		
Diaporthe longicolla	1		
Diaporthe perseae	1		
Diaporthe pseudomangiferae	4		
Diaporthe tectonae	1		

Table 1 Continued.

Endophyte	No. of isolates screened	No. of isolates surviving exposure to 100ºC	
		2 Hrs	4 Hrs
Diaporthe sp. 1	3		
Emericella nidulans	1	1	
Fusarium spp.	9	2	
Glomerella spp.	11		
Lasiodiplodia theobromae	3		
Monilia sp.	1		
Nigrospora sp.	1		
Nodulisporium spp.	10		
Penicillium pedernalense	1		
Penicillium sp. 1	6	1	
Pestalotiopsis sp.	10	6	5
Phomopsis heveicola	1		
Phyllosticta capitalensis	12		
Trichoderma spp.	2	2	
Total Positive Strains	**129**	**27**	**13**

Table 2 Thermotolerance of spores of mangrove leaf endophytes exposed to prolonged heating.

Endophyte	Host	Exposed to 100ºC		
		6 Hrs	8 Hrs	10 Hrs
Chaetomium globosum	*Acanthus ilicifolius*	+	+	–
Chaetomium globosum	*Avicennia officinalis*	+	+	–
Chaetomium globosum	*Bruguiera gymnorhiza*	–	–	–
Chaetomium globosum	*Rhizophora apiculata*	–	–	–
Chaetomium globosum	*Rhizophora stylosa*	+	+	+
Chaetomium sp. 3	*Rhizophora mucronata*	+	+	–
Chaetomium sp. 4	*Rhizophora apiculata*	–	–	–
Curvularia sp. 2	*Acanthus ebracteatus*	–	–	–
Pestalotiopsis sp.	*Bruguiera parviflora*	+	+	–
Pestalotiopsis sp.	*Ceriops tagal*	+	+	–
Pestalotiopsis sp.	*Nypa fruticans*	+	–	–
Pestalotiopsis sp.	*Rhizophora mucronata*	+	+	–
Pestalotiopsis sp.	*Sonneratia alba*	+	–	–
Total Positive Strains		**9**	**7**	**1**

– = No growth; + = growth observed

Table 3 Thermal resistance of ascospores of *C. globosum* in the presence of tricylazole.

Hours of treatment at 100ºC	Inhibitor (µg ml^{-1})			
	0	1	10	100
4	+	+	+	+
6	+	+	+	+
8	+	+	+	–
10	+	+	+	–

– = No growth; + = growth observed

Discussion

The higher temperature limit for the growth of eukaryotes is 60ºC (Tansey & Brock 1972). Thermal tolerance of fungal spores to wet heat is usually studied at temperatures below 60ºC since spores of many fungi do not survive exposure to this temperature for several minutes (Wyatt et al.

2013). According to van den Brule et al. (2020), the most heat resistant conidia are produced by a strain of *Paecilomyces variotii*, which could survive exposure to 60ºC for 22 minutes. Their work also emphasizes the importance of exposure time to heat since only 1% and 0.1% of the spores were alive after 44 and 66 minutes of treatment. In these and other studies (Bayne & Michener 1979), the test fungi are subjected to heat treatment when suspended in a medium. Our study focuses on dry heat tolerance where spores are subjected to rapid heating by transferring within a few seconds from their fully hydrated state to a heated dry oven (Suryanarayanan et al. 2011). Using this method, Suryanarayanan et al. (2011) showed that spores of mesophilic litter fungi from tropical dry forests experiencing episodic ground fires could tolerate rapid dry heating at 115ºC for 2 hours. They opine that such an extraordinary thermotolerance is the outcome of adaptations to prolonged droughts and the prevalence of fire in their dry habitat. The present study using endophytes of trees of mangrove vegetation reveals that this trait is not confined to fungi of dry habitats experiencing sporadic fires but is widespread. This is interesting since even the spores of thermophilic fungi survive exposure to 68ºC for 5–60 minutes only (Ogundero & Oso 1980) and 60ºC is the upper-temperature limit for these fungi (Maheshwari et al. 2000, Powell et al. 2012). We show here that the resting spores of some mesophilic fungi survive exposure to prolonged periods (2–10 hrs) to100ºC.

Since the fungi studied are from an environment not exposed to fire and the direct transfer from 22ºC to 100ºC is immediate, it is unlikely that the spores exhibit acquired thermotolerance shown by fungi when transferred from optimal temperature to high temperature in a stepwise manner (Tereshina 2005). The general method of thermal protection in spores adopted by fungi via the synthesis of compatible solutes such as mannitol and trehalose (Hagiwara et al. 2017) and heat shock proteins. This mechanism may not be involved here since dormant fungal spores are very low in their metabolic activities (Thevelein et al. 1984), including respiration (Novodvorska et al. 2016).

A few studies show that melanin pigment protects fungi from high temperatures (Butler & Day 1998, Gessler et al. 2014). The mycelia and spores of many of the endophytes of desert plants are pigmented (Suryanarayanan et al. 2005, Sangamesh et al. 2018). The presence of melanin pigment is known to afford protection to fungi against several abiotic stressors including heat (Ravishankar et al. 1995, Money et al. 1998, Paolo et al. 2006, Dadachova & Casadevall 2008, Geib et al. 2016). Spores of melanin deficient *Monilinia fructicola* are more sensitive to heat stress (Rehnstrom & Free 1996). Melanized *Cryptococcus neoformans* cells are less susceptible to heating at 42–47ºC than non-melanized cells (Rosas & Casadevall 1997). Melanized *Aureobasidium melanogenum* cells isolated from Taklimakan desert soil tolerated exposure to heat (40ºC) better than the non-melanized cells (Jiang et al. 2016). In the present study, ascospores of *C. globosum* showed very high thermotolerance. Since *C. globosum* synthesizes melanin (Hu et al. 2018), we proceeded to see if melanin could be responsible for such high thermotolerance in this fungus. Hu et al. (2012) reported that tricyclazole inhibits melanin synthesis in *Chaetomium globosum*, confirming that it is a 1,8-dihydroxynaphthalene (DHN) type of melanin. We found that the thermotolerance of *C. globosum* spores reduced when melanin synthesis was inhibited in cultures grown in the presence of the inhibitor, suggesting that melanin plays a critical role in the extraordinary thermal resistance of fungal spores. Although fungi like *Phyllosticta capitalensis* reported having DHN melanin (Suryanarayanan et al. 2004), none of the 12 isolates of this fungus had thermotolerant spores. Similarly, the heat tolerance observed here was not strictly taxon-specific as the same genus or even species isolated from different plants showed different degrees of thermotolerance (Tables 1, 2). A caveat about the methodology used in the present study should be noted. It involves the immediate transfer of spores from normal temperature to the heated environment under dry condition (without suspending the spores in a medium like water) and hence a spore count could not be made; thus, the number of spores subjected to heating is not known. Furthermore, the possibility of some hyphal bits getting transferred along with the spores cannot be ruled out. It is relevant that heat tolerance in *Saccharomyces cerevisiae* differs with isolates and is a complex polygenic trait (McCusker et al. 1994). Similar studies at the genome level are needed to

explain heat resistance trait difference among spores of the same species seen in the present study especially since information on the role of melanin in heat protection in fungi is scarce (Cordero & Casadevall 2017).

The result that spores of some endophytes like *Alternaria*, *Chaetomium*, *Fusarium* and *Pestalotiopsis* spp. are thermal resistant leads to some speculations. These leaf endophytes have a wide range of plant host and geographic distribution (Suryanarayanan et al. 2018) and are capable of shifting to a saprotrophic litter degrading mode once the leaf falls by elaborating biomass degrading enzymes (Prakash et al. 2015, Reddy et al. 2016). Their ecological amplitude could be enlarged further owing to heat resistant spores especially under the current global warming scenario (Sadyś et al. 2016). Furthermore, thermal resistant spores of plant pathogenic fungi survive longer in soils and could be a major concern since models predict that global warming could increase the relative abundance of plant pathogens worldwide (Delgado-Baquerizo et al. 2020). These observations, taken together with our study, underscore the importance of studying the performance of crop pathogenic fungi producing melanized resting structures (Velásquez et al. 2018). *Chaetomium* spp. are usually saprotrophic but some including *C. globosum* could cause mycoses including onychomycosis, sinusitis, empyema, pneumonia, and fatal cerebral disease in immunocompromised patients (Barron et al. 2003, Ahmed et al. 2016). Our demonstration that spores of *C. globosum* are extraordinarily heat-tolerant warrants more studies as they may not be inhibited by the natural barrier of higher temperature of the human body (Garcia-Solache & Casadevall 2010). Many species of the order Sordariales, to which *Chaetomium* belongs, are known to be thermophilic (Sandona et al. 2019) and grow better at 45°C (Morgenstern et al. 2012). Though thermophilic fungi, which exhibit growth in higher temperatures, have been studied well, the current study emphasizes the need to study thermal resistance of resting structures of fungi as well.

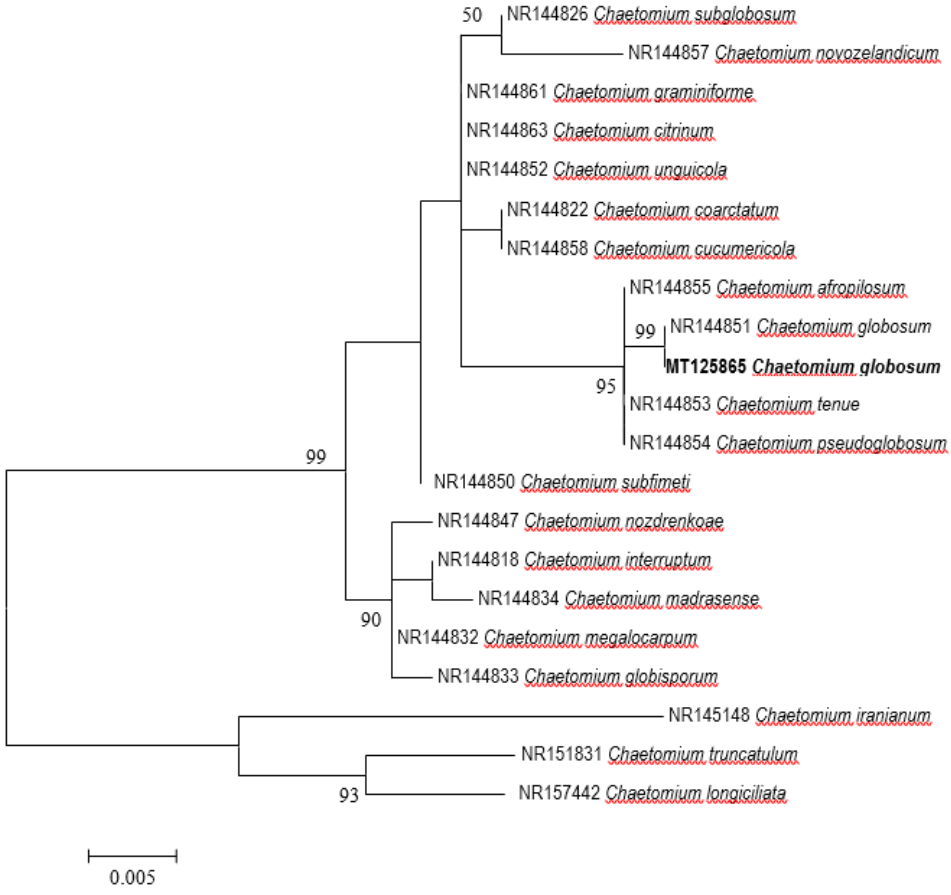

Fig. 1 – Maximum likelihood phylogenetic tree of *C. globosum*

Acknowledgements

We thank Prof. Nicholas P. Money, Dept. Biology, Miami University, Ohio for his valuable comments on a draft of the manuscript and Swami Shukadevananda, Secretary, Ramakrishna Mission Vidyapith, Chennai for facilities provided. TSS, MBG, and TR thank the Department of Biotechnology, Government of India for the grant of a research project (BT/PR7026/NDB/39/458/2013), SRF and JRF Fellowships respectively. TSS thanks the Principal Chief Conservator of Forests (PCCF), Andaman and Nicobar Islands for permitting the collection of leaf samples in the DBT research project.

References

Ahmed SA, Khan Z, Wang X-W, Moussa TAA et al. 2016 – *Chaetomium*-like fungi causing opportunistic infections in humans: a possible role for extremotolerance. Fungal Diversity 76, 11–26.

Barron MA, Sutton DA, Veve R, Guarro J et al. 2003 – Invasive mycotic infections caused by *Chaetomium perlucidum*, a new agent of cerebral phaeohyphomycosis. Journal of Clinical Microbiology 41, 5302–5307.

Bayne HG, Michener HD. 1979 – Heat resistance of *Byssochlamys* ascospores. Applied and Environmental Microbiology 37, 449–453.

Bell AA, Wheeler MH. 1986 – Biosynthesis and functions of fungal melanins. Annual Review of Phytopathology 24, 411–451.

Butler MJ, Day AW. 1998 – Fungal melanins: a review. Canadian Journal of Microbiology 44, 1115–1136.

Cooney DG, Emerson R. 1964 – Thermophilic fungi: An account of their biology, activities, and classification. W. H. Freeman & Co., San Francisco & London.

Cordero RJB, Casadevall A. 2017 – Functions of fungal melanin beyond virulence. Fungal Biology Reviews 31, 99–112.

Dadachova E, Casadevall A. 2008 – Ionizing radiation: how fungi cope, adapt, and exploit with the help of melanin. Current Opinion in Microbiology 11, 525–531.

Delgado-Baquerizo M, Guerra CA, Cano-Díaz C, Egidi E et al. 2020 – The proportion of soil-borne pathogens increases with warming at the global scale. Nature Climate Change 10, 550–554.

Ellis MB. 1971 – Dematiaceous Hyphomycetes. CMI, Kew, Surrey, U.K.

Ellis MB. 1976 – More Dematiaceous Hyphomycetes. CMI, Kew, Surrey, U.K.

Ellis MB, Ellis JP. 1988 – Microfungi on Miscellaneous Substrates: An Identification Handbook. Croom Helm Ltd., London, U.K.

Garcia-Solache MA, Casadevall A. 2010 – Hypothesis: global warming will bring new fungal diseases for mammals. mBio 1, e00061-10.

Geib E, Gressler M, Viediernikova I, Hillmann F, Jacobsen ID, Nietzsche S, Hertweck C, Brock M. 2016 – A non-canonical melanin biosynthesis pathway protects *Aspergillus terreus* conidia from environmental stress. Cell Chemical Biology 23, 587–597.

Gessler NN, Egorova AS, Belozerskaia TA. 2014 – Melanin pigments of fungi under extreme environmental conditions (Review). Applied Biochemistry and Microbiology 50, 105–113.

Hagiwara D, Sakai K, Suzuki S, Umemura M et al. 2017 – Temperature during conidiation affects stress tolerance, pigmentation, and trypacidin accumulation in the conidia of the airborne pathogen *Aspergillus fumigatus*. PLoS ONE 12, e0177050.

Hu Y, Hao XR, Chen L, Akhberdi O, et al. 2018 – Gα-cAMP/PKA pathway positively regulates pigmentation, chaetoglobosin A biosynthesis and sexual development in *Chaetomium globosum*. PLoS ONE 13, e0195553.

Hu Y, Hao XR, Lou J, Zhang P et al. 2012 – A PKS gene, *pks-1*, is involved in chaetoglobosin biosynthesis, pigmentation and sporulation in *Chaetomium globosum*. Science China Life Sciences 55, 1100–1108.

Jiang H, Liu N-N, Liu G-L, Chi Z et al. 2016 – Melanin production by a yeast strain XJ5-1 of *Aureobasidium melanogenum* isolated from the Taklimakan desert and its role in the yeast survival in stress environments. Extremophiles 20, 567–577.

Kimura M. 1980 – A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16, 111–120.

Maheshwari R, Bharadwaj G, Bhat MK. 2000 – Thermophilic fungi: their physiology and enzymes. Microbiology and Molecular Biology Reviews 64, 461–488.

McCusker JH, Clemons KV, Stevens DA, Davis RW. 1994 – Genetic characterization of pathogenic *Saccharomyces cerevisiae* isolates. Genetics 136, 1261–1269.

Money NP, Caesar-TonThat T-C, Frederick B, Henson JM. 1998 – Melanin synthesis is associated with changes in hyphopodial turgor, permeability, and wall rigidity in *Gaeumannomyces graminis var. graminis*. Fungal Genetics and Biology 24, 240–251.

Morgenstern I, Powlowski J, Ishmael N, Darmond C et al. 2012 – A molecular phylogeny of thermophilic fungi. Fungal Biology 116, 489–502.

Novodvorska M, Stratford M, Blythe MJ, Wilson R et al. 2016 – Metabolic activity in dormant conidia of *Aspergillus niger* and developmental changes during conidial outgrowth. Fungal Genetics and Biology 94, 23–31.

Ogundero VW, Oso BA. 1980 – Thermal resistance and viability of asexual spores of thermophilic fungi from composts. Zeitschrift für allgemeine Mikrobiologie 20, 513–516.

Onions AHS, Allsopp D, Eggins HOW. 1981 – Smith's Introduction to Industrial Mycology. VII ed. Edward Arnold, London, U.K.

Paolo WF, Dadachova E, Mandal P, Casadevall A et al. 2006 – Effects of disrupting the polyketide synthase gene *WdPKS1* in *Wangiella* [*Exophiala*] *dermatitidis* on melanin production and resistance to killing by antifungal compounds, enzymatic degradation, and extremes in temperature. BMC Microbiology 6, 55.

Powell AJ, Parchert KJ, Bustamante JM, Ricken JB et al. 2012 – Thermophilic fungi in an aridland ecosystem. Mycologia 104, 813–825.

Prakash CP, Thirumalai E, Govinda Rajulu MB, Thirunavukkarasu N, Suryanarayanan TS. 2015 – Ecology and diversity of leaf litter fungi during early-stage decomposition in a seasonally dry tropical forest. Fungal Ecology 17, 103–113.

Rajamani T, Suryanarayanan TS, Murali TS, Thirunavukkarasu N. 2018 – Distribution and diversity of foliar endophytic fungi in the mangroves of Andaman Islands, India. Fungal Ecology 36, 109–116.

Ravishankar JP, Muruganandam V, Suryanarayanan TS. 1995 – Isolation and characterization of melanin from a marine fungus. Botanica Marina 38, 413–416.

Reddy MS, Murali TS, Suryanarayanan TS, Govinda Rajulu MB, Thirunavukkarasu N. 2016 – *Pestalotiopsis* species occur as generalist endophytes in trees of Western Ghats forests of southern India. Fungal Ecology 24, 70–75.

Rehnstrom AL, Free SJ. 1996 – The isolation and characterization of melanin-deficient mutants of *Monilinia fructicola*. Physiological and Molecular Plant Pathology 49, 321–330.

Rosas ÁL, Casadevall A. 1997 – Melanization affects susceptibility of *Cryptococcus neoformans* to heat and cold. FEMS Microbiology Letters 153, 265–272.

Sadyś M, Kennedy R, West JS. 2016 – Potential impact of climate change on fungal distributions: analysis of 2 years of contrasting weather in the UK. Aerobiologia 32, 127–137.

Sandona K, Billingsley Tobias TL, Hutchinson MI et al. 2019 – Diversity of thermophilic and thermotolerant fungi in corn grain. Mycologia 111, 719–729.

Sangamesh MB, Jambagi S, Vasanthakumari MM, Shetty NJ et al. 2018 – Thermotolerance of fungal endophytes isolated from plants adapted to the Thar Desert, India. Symbiosis 75, 135–147.

Sawmya K, Vasudevan TG, Murali TS. 2013 – Fungal endophytes from two orchid species – pointer towards organ specificity. Czech Mycology 65, 89–101.

Suryanarayanan TS, Devarajan PT, Girivasan KP, Govinda Rajulu MB et al. 2018 – The host range of multi-host endophytic fungi. Current Science 115, 1963–1969.
Suryanarayanan TS, Govinda Rajulu MB, Thirumalai E, Reddy MS, Money NP. 2011 – Agni's fungi: heat-resistant spores from the Western Ghats, southern India. Fungal Biology 115, 833–838.
Suryanarayanan TS, Wittlinger SK, Faeth SH. 2005 – Endophytic fungi associated with cacti in Arizona. Mycological Research 109, 635–639.
Suryanarayanan TS, Ravishankar JP, Venkatesan G, Murali TS. 2004 – Characterization of melanin pigment of a cosmopolitan fungal endophyte. Mycological Research 108, 974–978.
Sutton BC. 1980 – The Coelomycetes: Fungi Imperfecti with Pycnidia, Acervuli and Stromata. CMI, Kew, Surrey, U.K.
Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013 – MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30, 2725–2729.
Tansey MR, Brock TD. 1972 – The upper temperature limit for eukaryotic organisms. Proceedings of the National Academy of Sciences of the USA 69, 2426–2428.
Tereshina VM. 2005 – Thermotolerance in fungi: the role of heat shock proteins and trehalose. Microbiology 74, 247–257.
Thevelein JM, den Hollander JA, Shulman RG. 1984 – Trehalase and the control of dormancy and induction of germination in fungal spores. Trends in Biochemical Sciences 9, 495–497.
Thompson JD, Higgins DG, Gibson TJ. 1994 – CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22, 4673–4680.
van den Brule T, Punt M, Teertstra W, Houbraken J et al. 2020 – The most heat-resistant conidia observed to date are formed by distinct strains of *Paecilomyces variotii*. Environmental Microbiology 22, 986–999.
Velásquez AC, Castroverde CDM, He SY. 2018 – Plant–pathogen warfare under changing climate conditions. Current Biology, 28, R619–R634.
White TJ, Bruns T, Lee S, Taylor J. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR Protocols: A Guide to Methods and Applications. San Diego: Academic Press, pp. 315–322.
Wyatt TT, Wösten HAB, Dijksterhuis J. 2013 – Fungal spores for dispersion in space and time. Advances in Applied Microbiology 85, 43–91.