Vertebrate Endothermy Restricts Most Fungi as Potential Pathogens

Vincent A. Robert[1] and Arturo Casadevall[2]

[1]Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; [2]Department of Microbiology and Immunology and Division of Infectious Diseases, Department of Medicine, Albert Einstein College of Medicine, Yeshiva University, Bronx, New York

The paucity of fungal diseases in mammals relative to insects, amphibians, and plants is puzzling. We analyzed the thermal tolerance of 4802 fungal strains from 144 genera and found that most cannot grow at mammalian temperatures. Fungi from insects and mammals had greater thermal tolerances than did isolates from soils and plants. Every 1°C increase in the 30°C–40°C range excluded an additional 6% of fungal isolates, implying that fever could significantly increase the thermal exclusion zone. Mammalian endothermy and homeothermy are potent nonspecific defenses against most fungi that could have provided a strong evolutionary survival advantage against fungal diseases.

Of the 1.5 million fungal species, only a few hundred are pathogenic to mammals [1]. Fungal diseases in mammals often reflect impaired immune function, and fungi did not emerge as major pathogens for humans until the late 20th century. For example, candidiasis was uncommon until the 1950s, when thrush was associated with the introduction of antibiotics that disrupted bacterial flora. Similarly, diseases such as cryptococcosis, aspergillosis, and histoplasmosis were rare until recently, when their prevalence increased with the human immunodeficiency virus epidemic and the development of immunosuppressive therapies. In contrast, the number of fungal species pathogenic to plants and insects is estimated to be 270,000 and 50,000, respectively [2]. Amphibians are particularly vulnerable to certain fungal infections, as evidenced by the current catastrophic epidemic of chytridiomycosis in frogs.

The resistance of mammals with intact immune systems to systemic fungal diseases, coupled with their endothermic and homeothermic lifestyles, suggested that these costly physiological adaptations were evolutionarily selected because they conferred a survival advantage by protecting against environmental pathogens [3]. However, testing this hypothesis was difficult because knowledge of fungal thermal tolerance is largely anecdotal. Consequently, we evaluated the thermal growth tolerances of fungal species in a reference collection and compared them to mammalian temperatures.

Methods. A total of 4802 fungal strains belonging to 144 genera in the Centraalbureau voor Schimmelcultures (Utrecht) collection were tested for growth at 4°C, 12°C, 15°C, 18°C, 21°C, 25°C, 30°C, 35°C, 37°C, 40°C, 42°C, and 45°C. Strains were grown for times ranging from a few days to a few weeks on the most suitable medium, generally glucose–peptone–yeast extract agar, potato-dextrose agar, or yeast extract–malt extract agar. Growth was considered positive when a colony was visible without magnification. The strain set included Ascomycetes and Basidiomycetes but excluded Zygomycetes, which is not in the yeast database.

The culture deposit records were reviewed to identify the isolation source. Fungi isolated from flowers, grains, and herbal exudates were grouped under plant isolates. Animal isolates were classified depending on whether they originated from endothermic (mammals and birds) or ectothermic (insects, nematodes, fishes, and crustaceans) species. Another group comprised isolates from nonliving environmental sources, which included predominantly soils; this group is referred to as soil isolates. These groups were compared for thermal tolerance at 2 temperatures, 25°C and 37°C, which reflect ambient and mammalian temperatures, respectively.

To test the significance of the difference in growth patterns between fungal strains isolated from different groups, we calculated the test statistics

$$z = (p^1 - p^2)/\sqrt{P \times (1 - P) \times (1/n^1 + 1/n^2)} \ ,$$

where p^1 and p^2 are the observed sample proportions for each group of fungal strains at a given temperature, n^1 and n^2 are the size of the 2 groups under comparison, and

$$P = (p^1 \times n^1 + p^2 \times n^2)/(n^1 + n^2) \ .$$

Received 23 May 2009; accepted 18 June 2009; electronically published 14 October 2009.
Potential conflicts of interest: none reported.
Financial support: National Institutes of Health (awards 5R01AI033774, 5R01HL059842, and 2U54AI057158).

Reprints or Correspondence: Dr Arturo Casadevall, Department of Medicine, Albert Einstein College of Medicine, Yeshiva University, 1300 Morris Park Ave, Bronx, NY 10461 (arturo.casadevall@einstein.yu.edu).

The Journal of Infectious Diseases 2009; 200:1623–6

0022-1899/2009/20010-0020$15.00
DOI: 10.1086/644642

The statistic z was assumed to be distributed normally. The 2-tailed probability from the absolute z score to infinity on both tails of the distribution was calculated (http://www.danielsoper.com/statcalc/calc21.aspx) and confirmed using the NORMSDIST function in Excel (Microsoft) to assess the significance of differences in growth between groups at different temperatures.

Results and Discussion. Knowledge of fungal thermal tolerance is limited to a few species because the subject has not been systematically studied. In fact, such studies may be very difficult to do, and a comprehensive prospective study of fungal thermal tolerance would require a gargantuan effort. However, culture collections provide an attractive alternative for initial explorations of this subject. Culture collections store and maintain fungal strains and record basic nutritional needs and temperature tolerances. This information, when accessed and analyzed with bioinformatics tools, provides a useful starting point for the analysis of fungal thermal tolerances.

Our results show that most strains grew well in the 12°C–30°C range, but there was a rapid decline in thermal tolerance at temperatures >35°C (Figure 1). A plot of the fraction of fungal strain that grew versus temperature in the 30°C–42°C range revealed a linear relationship with an equation of $y = -0.0166x \times 2.7911$, such that for every 1° increase in temperature >30°C, ~6% fewer strains could grow.

For 3020 strains, there was information on both source isolation and temperature tolerance. This group included isolates from the environment (primarily soils), plants, ectothermic animals, and endothermic animals. The majority of these isolates grew at 25°C regardless of their source (Table 1). Nevertheless, the proportion growing at 25°C was significantly greater for isolates recovered from living hosts than from soils, irrespective of whether the hosts were ectothermic plants and animals or endothermic animals. At 37°C, the proportion of fungi that grew was much higher for isolates from endothermic animals than from ectothermic animals. The proportions of Ascomycetes and Basidiomycetes fungi in each group were comparable, except for ectothermic hosts, which yielded predominantly Ascomycetes fungi.

Isolates from ectothermic hosts (such as plants and insects) were significantly more thermotolerant than isolates from soils. A significantly greater percentage of fungal strains from insects grew at 37°C relative to those recovered from plants, possibly reflecting the fact that insects can increase their temperature through behavioral fevers that increase survival after fungal infection [5]. However, this explanation is unlikely to apply to plants, which have much lower metabolic rates. Since thermal tolerance must be associated with numerous metabolic changes that mitigate fungal damage, the association between greater thermotolerance and plant pathogenicity could mirror adaptation to survival in a host with potent antifungal defenses,

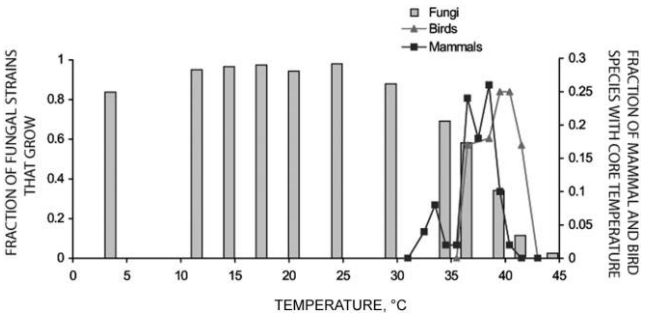

Figure 1. Frequency histogram of thermal growth tolerance for 4802 fungal strains (*bars*). Lines connect percentages for 49 mammalian (*blue*) and 12 bird (*red*) species core temperatures. Obtained from McNab [4].

raising the tantalizing possibility that selection pressures by virulence may contribute to thermal stability and vice versa. In this regard, we note that Hsp90 orchestrates morphogenesis in *Candida albicans* [6], thus providing a molecular association for heat shock and a virulence-related phenotype that may be conserved in other fungi.

A survey of the fungal genera represented in our sample collection revealed differences in the percentage of isolates capable of growth at 37°C. All genera studied included some thermotolerant species, as defined by their ability to grow at 37°C, but there were large differences in the percentage of species within each genera. Thermotolerant genera included those from both Ascomycetes and Basidiomycetes, but basidiomycetous genera were disproportionately more common among the thermotolerant genera ($P < .001$, Fisher exact test). The strains grouped within the sexually related basidiomycetous genera *Filobasidiella* (a telemorph of *Cryptococcus*) and *Cryptococcus* (an anamorph of *Filobasidiella*) included comparable numbers of thermotolerant species (61% among 116 strains and 53% among 287 strains, respectively). These data suggest an association between phylogeny and thermotolerance.

The capacity for thermotolerance was interspersed among Ascomycetes and Basidiomycetes, suggesting that it may have emerged independently several times in evolution. Alternatively, thermotolerance may be an ancient fungal trait that was lost by those species that cannot grow at 37°C. In this regard, we note that the climate for much of Earth's history was much warmer than in recent geologic epochs, having cooled by ~5°C during the Eocene-Oligocene transition ~34 million years ago [7]. The fact that thermotolerance is a complex trait that can be lost by a single mutation, as demonstrated by laboratory-generated temperature-sensitive mutants, makes the explanation of a retained phenotype attractive.

Our results may be relevant to the ongoing debate on the origin and function of endothermy, homeothermy, and fever, each a major unsolved problem in vertebrate physiology [8, 9]. There is no consensus as to why mammals have adopted such

Table 1. Growth Tolerances for Fungi from Soils, Animals, and Plants at 2 Temperatures

Origin, host type	Isolate Growth			Total	*P* values[b]		
	Yes	No	Unknown[a]		*P*1	*P*2	*P*3
	at 25°C						
Soils, NA	657	42	7	706			
Plant, ectotherm	1108	30	5	1143	<.001		
Animal							
Ectotherm	490	0	6	496	<.001	.029	
Endotherm	661	5	9	675	<.001	.214	.263
	at 37°C						
Soils, NA	146	535	15	706			
Plant, ectotherm	304	871	22	1143	.292		
Animal							
Ectotherm	193	284	19	496	<.001	.004	
Endotherm	466	202	7	675	<.001	<.001	<.001

NOTE. NA, not applicable.

[b] *P*1 refers to the comparison of isolates from soils, *P*2 refers to the comparison versus plant isolates, and *P*3 refers to the comparison between isolates from ectothermic and endothermic animals .

[a] Refers to a small no. of isolates for which the temperature growth data was not complete.

an energetically costly lifestyle. Endothermy is associated with certain metabolic benefits and thermodynamic efficiency, but these benefits come at a high cost since endothermic vertebrates require ~10 times more oxygen to support metabolism than do ectothermic vertebrates [8]. Our analysis suggests that part of the cost is mitigated by the creation of a thermal exclusionary zone that can protect against environmental microbes. Given the high metabolic cost of endothermy, the core temperatures of individual mammal and bird species are likely to be a compromise between its benefits and costs. If endothermy was selected for protection against infectious disease, then a case could be made that endothermy preceded homeothermy. Similarly, if one considers fever as a mechanism to extend the thermal exclusionary zone against environmental microbes such as fungi, increases in temperature of only 1°–3° can significantly reduce the proportion of such microbes that can inhabit the host.

The benefits of endothermy and homeothermy in protection against microbes do not appear to have been previously considered as mechanisms for evolutionary selection, possibly because most of the viral and bacterial diseases that currently plague animals are often acquired from other warm hosts, and these necessarily involve thermotolerant microbes. However, the perspective is very different when one focuses on environmentally acquired microbes and the fungi in particular. Pathogenic microbes are a very small subset of the total terrestrial microbial flora, and these can be divided as to whether they are acquired from other hosts or directly from the environment [10]. For mammals, pathogenic microbes acquired from other hosts are usually adapted to mammalian temperatures, but microbes acquired directly from the environment would not be subject to such selection pressures. Hence, the potential benefit of endothermy and homeothermy to host defense may become apparent only when one considers the entire microbiota and that subset of pathogenic microbes that is acquired directly from the environment. In support of this notion, we note that bats become susceptible to a cold-loving fungus when hibernation greatly reduces their body temperatures [11] and that primitive mammals (such as the egg-laying platypus, which has a body temperature of 32°C) are susceptible to fungal diseases [12]. An epidemiological observation consistent with the protective function of endothermy comes from the observation that serotype D *Cryptococcus neoformans* are less thermotolerant [13] than other varieties and are associated with cutaneous cryptococcosis [14]. Experimental support for the notion that endothermy restricts fungal infection comes from the observation that rabbits, which have core temperatures of 38°C–39°C, are notoriously resistant to cryptococcosis, and infection can be induced only in cooler organs, such as testes [15]. However, the same system also shows that mammalian immune systems also make a decisive contribution to host defense against fungi since systemic cryptococcosis can be induced in rabbits after corticosteroid administration [15].

This study reflects the power of a bioinformatics analysis of archival data from culture collection, which allows comparison of temperature growth data on thousands of isolates. However, there are certain limitations that should be considered in evaluating the data. Strains from plants and insects were disproportionately represented in the collection, and this may introduce certain biases. The relative paucity or absence of strains from certain sources and taxonomic groups could contribute to bias in the statistical analysis. For example, there were rel-

atively few isolates from birds and ectothermic animals other than insects, no Zygomycetes fungi, and only a few filamentous fungi. Furthermore, the catalogued information was insufficiently detailed to distinguish between skin and systemic isolates from endotherms, which could differ in thermotolerance.

The discovery of fossilized fungal proliferation at the Cretaceous-Tertiary boundary was proposed to contribute to extinction events at the end of the Cretaceous epoch that replaced reptiles with mammals as the dominant large animals [3]. Thermal tolerance is a necessary, but not sufficient, characteristic of microbes being capable of causing invasive disease in mammals. Since thermal tolerance almost certainly involves many genes and biochemical processes, it is unlikely that this trait can be rapidly acquired by any one microbial species. Consequently, new human pathogenic fungi are likely to emerge from genera that are already tolerant to higher temperatures; such species may warrant special attention given likely climatic changes in the years ahead that could alter patterns of fungal prevalence.

References

1. Kwon-Chung KJ, Bennett JE. Medical mycology. Philadelphia: Lea & Febiger, **1992**.
2. Hawksworth DL, Rossman AY. Where are all the undescribed fungi? Phytopathology **1997**; 87:888–91.
3. Casadevall A. Fungal virulence, vertebrate endothermy, and dinosaur extinction: is there a connection? Fungal Genet Biol **2005**; 42:98–106.
4. McNab BK. Body weight and the energetics of temperature regulation. J Exp Biol **1970**; 53:329–48.
5. Thomas MB, Blanford S. Thermal biology in insect-pathogen interactions. Trends Ecol Evol **2003**; 18:344–50.
6. Shapiro RS, Uppuluri P, Zaas AK, et al. Hsp90 orchestrates temperature-dependent *Candida albicans* morphogenesis via Ras1-PKA signaling. Curr Biol **2009**; 19:621–9.
7. Liu Z, Pagani M, Zinniker D, et al. Global cooling during the eocene-oligocene climate transition. Science **2009**; 323:1187–90.
8. Ruben J. The evolution of endothermy in mammals and birds: from physiology to fossils. Annu Rev Physiol **1995**; 57:69–95.
9. Kemp TS. The origin of mammalian endothermy: a paradigm for the evolution of complex biological structure. Zool J Linn Soc **2008**; 147: 473–88.
10. Casadevall A, Pirofski LA. Accidental virulence, cryptic pathogenesis, martians, lost hosts, and the pathogenicity of environmental microbes. Eukaryot Cell **2007**; 6:2169–74.
11. Blehert DS, Hicks AC, Behr M, et al. Bat white-nose syndrome: an emerging fungal pathogen? Science **2009**; 323:227.
12. Obendorf DL, Peel BF, Munday BL. *Mucor amphibiorum* infection in platypus (*Ornithorhynchus anatinus*) from Tasmania. J Wildl Dis **1993**; 29:485–7.
13. Martinez LR, Garcia-Rivera J, Casadevall A. *Cryptococcus neoformans* var. *neoformans* (serotype D) strains are more susceptible to heat than *C. neoformans* var. *grubii* (serotype A) strains. J Clin Microbiol **2001**;39: 3365–7.
14. Dromer F, Mathoulin S, Dupont B, Letenneur L, Ronin O. Individual and environmental factors associated with infection due to *Cryptococcus neoformans* serotype D. Clin Infect Dis **1996**; 23:91–6.
15. Perfect JR, Lang SDR, Durack DT. Chronic cryptococcal meningitis. Am J Path **1980**; 101:177–93.