Hypothermia is a characteristic of the fungal kingdom

Radames JB Cordero and Arturo Casadevall

Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, 615 North Wolfe Street, Baltimore, MD 21205, USA

* acasade1@jhu.edu

Email: acasade1@jhu.edu

RJBC: 0000-0002-3026-7094; AC: 0000-0002-9402-9167

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RC conceived the original idea, carried out the experiments, and wrote the manuscript with support from AC. AC supervised the project.

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Abstract

Fungi play essential roles in global ecology and economy, but their thermal biology is widely unknown. Infrared imaging revealed that mushrooms, yeasts, and molds each maintained colder temperatures than their surroundings. Fungal specimens are to be ~2.5 °C colder than the surrounding temperature. Time-lapse infrared images of *Pleurotus ostreatus* revealed hypothermia throughout mushroom growth and after detachment from mycelium. The hymenium was coldest, and different areas of the mushroom exhibit distinct thermal changes during heating and cooling. The fruiting area in the mycelium remained relatively cold following mushroom detachment. Analyses of *Agaricus bisporus* mushroom pilei confirmed that the mechanism for mushroom hypothermia depends on evaporative cooling. We also assessed evaporative cooling in biofilms of *Cryptococcus neoformans*, and *Penicillium* spp. molds based on the accumulation of condensed water droplets on the lids over biofilms grown on agar media plates. Biofilms of *C. neoformans* acapsular mutant showed more transpiration and were colder than wildtype. *Penicillium* biofilms appear to transpire ten times more than the supporting agar. We used the evaporative cooling capacity of mushrooms to construct a mushroom-based air-cooling system (MycoCooler™) capable of passively reducing the temperature of a closed compartment by approximately 10 °C in 25 minutes. This study suggests that hypothermia is a characteristic of the fungal kingdom. Since fungi make up ~2% of Earth biomass, their ability to dissipate heat may contribute significantly to planetary temperatures in local environments. These findings are relevant to the current global warming crisis and suggest that large-scale myco-cultures could help mitigate increasing planetary temperature.

Introduction

Temperature controls the growth, reproduction, and dispersal of all life forms. The temperature of an organism depends on the balance between gaining and dissipating heat as it is influenced by its total environment (i.e., physical-chemical, biotic-abiotic, micro-macro dimensions) (1). In theory, if the organism gains more thermal energy than it dissipates, it becomes warmer. If more thermal energy is lost, the organism may reach colder temperatures than its surroundings. When the organism and the environment each have the same temperature, there is no heat flow; hence the organism is in thermal equilibrium. Living organisms are considered dissipative systems that exist far from thermodynamic equilibrium (2); that could mean warmer or colder, but not equal to the surroundings.

Organisms can be classified based on their capacity to maintain their body temperatures relative to their environment. Endothermic organisms (alias 'warm-blooded'), like birds and mammals, can maintain relatively constant internal temperatures that range from 36 to 40 ºC, regardless of any fluctuations in outside temperature. Most life forms are, however, *ectothermic* (alias 'cold-blooded') because their internal temperatures fluctuate based on external temperatures. Much of the energy driving ectotherm metabolism comes from their surroundings, capturing heat from radiation energy via pigments like melanin (1). Plants makeup ~80% of Earth biomass (3) and may be considered the "epitome of poikilothermy" because these are frequently found in environments that are subject to wide variations in temperature and, contrary to reptiles or fish, cannot displace to more favorable thermal environments when needed (4). To prevent overheating, plants and animals, give off heat via the evaporation of water at their surfaces in a process known as evaporative cooling, transpiration, or evapotranspiration. The evaporation of water is an endothermic process that consumes thermal energy to break hydrogen bonds when water goes from liquid to a gas. Cellular structures like animal sweat glands and plant stomas regulate the water transpiration process. Depending on the thermal environmental conditions, leaves can dissipate heat via evaporative cooling and become colder than air temperature (5–7). Fungal, protist, archaeal, and bacterial communities are assumed to be ectothermic considering their relatively simpler physiology and small size or high surface area-volume ratio, however, the temperature of microbial communities and the mechanisms of heat exchange with their surroundings are unknown.

In geologic history, the fungi pioneered the colonization of land and today play a central role in balancing Earth's ecology by breaking down decaying biological matter and providing nutrients for new growth. Fungal organisms can survive almost anywhere and are a source of food, medicines, and a variety of biomaterials. Fungi come in the forms of microscopic to macroscopic mushroom-producing mycelium, yeasts, and molds communities. The fungal kingdom also includes species that are pathogenic to animal and plant flora, causing severe public health and agricultural problems. Mushrooms, the reproductive structure of fungal mycelium, are usually formed by a stem or stalk and a cap or pileus. Pilei are often convex but can also form other shapes during development and between species. The area underneath the pilei, the hymenium, consists of lamellae gills or porous surfaces bearing spores. The

lamellar constitution of the hymenium can increase the surface area of mushrooms by 20 folds (8). The structural organization of the hymenium is important for spore production and spore release.

Mushroom pilei were noted to be cold relative to their surroundings (9–12). The first study inserted thermocouple detectors into mushrooms and suggested that the relatively cold temperatures were mediated by evaporative cooling (11). Quantitative data of mushroom transpiration was provided in subsequent studies (9, 10). Mahajan et al. quantified the transpiration rate of *A. bisporous* whole mushrooms and developed a mathematical model to link mushroom water loss with ambient temperature and relative humidity (9). Subsequently, Dressaire et al. quantified the rate of water loss from mushroom pilei, which can be higher than plants and enough to cool the surrounding air by several degrees Celsius (10).

In this study, we applied infrared imaging to measure the temperature of wild mushrooms, as well as molds and yeasts biofilms/colonies under a variety of conditions. Our findings extend the observation that fungi are hypothermic to unicellular organisms through a common mechanism that involves cooling from the evaporation of fungal-associated water.

Results

Mushrooms, yeasts, and molds maintain colder temperatures than their surroundings. Thermal imaging of 21 wildlife mushroom species revealed that each was colder than their natural environment (**Fig. 1 a-f** and **Table S1**). The temperature of stalks recorded for some wild specimens was similar to the pilei. Yeast colonies and mold biofilms of *Candida* spp., *Cladosporium* spp., *Penicillium* spp., and *Rhodotorula mucilagenosa* also exhibited lower temperatures than the surrounding agar media following 1 h incubation at 45 °C (**Fig. 1 g-j**). The temperature differences between the fungus and its surroundings averaged ~2.5 °C and varied from ~0.5 to 5 °C, depending on the fungal specimen (**Fig. 1 k**). The mushrooms of *P. ostreatus* and *Cerrena unicolor* showed larger temperature differences; ~5 °C cooler than ambient temperature. The temperatures of all fungal specimens correlated linearly with surrounding temperatures at a slope of 1 and x, y-intercepts of ~2 °C (**Fig S1**).

Change in mushroom temperature during fruiting, heating, and cooling. *Pleurotus ostreatus* grown in the laboratory at 25 °C revealed coldness throughout the whole fruiting process (**Fig. 2 a**). We recorded colder temperatures over time, as the mushroom flush grew in size. The mushroom flush remained relatively cold after detachment, although several degrees warmer. The gills area underneath the pileus or hymenium appeared colder than the frontal side of *P. ostreatus* pilei or stalk (**Fig. 2 b**). Notably, the fruiting site of the mycelium also remained relatively cold after mushroom detachment, approximately 2.5 °C cooler than the rest (**Fig. 2 b**). The relatively cold temperature of the *P. ostreatus* mushroom was maintained during heating, increasing from approximately 19 to 27 °C following 137 minutes incubation at 37 °C (<10 % relative humidity, RH) (**Fig. 2 c**). After heating, the mushroom flush was incubated at 4 °C (<10 % RH), and its temperature dropped from 24 to 18 °C after 104 minutes (**Fig. 2 d**). A comparison of the thermal images of the mushroom flush during heating and cooling incubations showed that different areas of the mushroom dissipate heat differently. The changes in mushroom temperature during cooling manifested more irregular thermal gradients when compared to the heating incubation (**Fig S2**). The change in average mushroom temperature as a function of time followed an exponential curve during heating but a linear curve during cooling (**Fig S3**).

Fungal hypothermia is mediated by evaporative cooling. Evaporative cooling was confirmed in light and dark *A. bisporus* mushroom pilei by manipulating its water content and ambient temperature-humidity. Dehydrated mushrooms are no longer able to maintain relative colder temperatures, irrespective of ambient temperature (**Fig S4 a-c** and **Table S2)**. We observed similar temperature changes between light and dark mushrooms pilei. The percent mass loss of light and dark *A. bisporus* mushroom pilei following dehydration was 93.6 ± 0.4 % w/w (**Table S3**), demonstrating their high-water content. Dehydration of seven additional wild fungal unidentified specimens also shows high water content ranging from 57 to 92 percent by mass (**Table S3**). Mushrooms warmed slower and reached lower absolute temperatures under a dry environment as compared to a humid environment (**Fig**

S4 d&e). Together these results confirmed that mushroom's relative coldness was mediated by evaporative cooling.

Evaporative cooling in yeast and molds was evident from the condensation of water droplets on the lids above *Cryptococcus neoformans* and *Penicillium* spp. biofilms were grown upright on agar plates (**Fig. 3**). An acapsular mutant of *C. neoformans* showed more and larger water droplets than the encapsulated wildtype strain (**Fig. 3 a&b**). The encapsulated *C. neoformans* colonies are ~90% water, while the acapsular mutant is ~82% (**Table S3**). The mutant strain was also ~1 °C colder than the encapsulated strain. Biofilms of *Penicillium* spp. showed significant condensation of water droplets (**Fig. 3c**), at least ~10 times higher than the surrounding 1.5% agar medium (**Table S4**).

A mushroom-based air-cooling device. Figure 4 a show a diagram of a mushroom-based air-cooling device. We called this prototype device MycoCooler™, which was constructed using a Styrofoam box with a 1-cm diameter inlet aperture and a 2-cm diameter outlet aperture (**Fig S5 a**). An exhaust fan was attached outside the outlet aperture to drive airflow in and out of the box (**Fig. S5 b**). The MycoCooler™ was loaded with ~420 grams of *A. bisporus* mushrooms, closed, and placed inside a larger Styrofoam box (**Fig S5 c**) previously equilibrated inside a warm room (37 °C, <10% RH). Forty minutes after the addition of mushrooms, the temperature inside the closed Styrofoam box decreased approximately 10 °C at ~0.4 °C per min, and the humidity increased to ~45% at 1.3 % per min (**Fig 4 b**). While the humidity continued to increase, the air temperature reached a minimum at ~60% RH, at which point it started to increase back to initial temperature values (**Fig 4 b**). From this data, we estimated that 420 grams of *A. bisporus* mushroom pilei have an air-cooling capacity of approximately 20 Watts or 68 BTH/hr. The change in air temperature was proportional to change in humidity, confirming evaporative cooling as the mechanism for mushroom hypothermia. Our MycoCooler device provides a proof-of-principle for harnessing mushroom's cooling capacity for cooling air in enclosed environments.

Discussion

This thermographic study reveals that mushrooms, molds, and yeast can maintain colder temperatures than their environment, implying that hypothermia is a general property of the fungal world. Mushroom coldness occurred throughout the fruiting process, and the fruiting area of mycelium also became relatively cold. We also confirm that mushroom coldness occurs via transpiration and that this process also occurs in mold and yeast biofilms. Finally, we provide a proof-of-principle demonstration for a mushroom-based air-conditioning device capable of passively cooling and humidifying the air of a closed environment. The data presented here reveal the cold nature of fungal biology and evaporative cooling as a microbiological mechanism of thermoregulation.

The observation that fungal temperatures correlated to ambient temperature are consistent with the notion that fungi are poikilotherms. The cold temperatures of wild mushroom specimens relative to ambient temperature suggest that mushrooms are very effective at dissipating heat. The temperature of wild mushroom pilei varied between specimens, which suggests that there are species-specific capacities to dissipate heat that must be related to differences in still unknown thermal properties (i.e., heat capacity, thermal conductivity). The relatively cold temperatures of yeast colonies and mold biofilms were only visible after incubation in a warm/dry environment. At steady states in ambient temperature, our infrared imaging contrasting resolution was not sufficient to detect temperature differences between the colony/biofilm and the surrounding agar. The temperature difference becomes apparent as yeast colonies and mold biofilms can dissipate more heat than the surrounding agar, which is ~98 % water. Although we could not find any examples, we do not rule out the existence of mushrooms, yeasts, or molds capable of reaching warmer temperatures than their surroundings. Factors such as pigmentation and radiation exposure can influence fungal temperatures. Unicellular yeasts and mushrooms produce pigments, such as melanins, that can increase heat capture from radiation energy (13–15). For instance, approximately 1 gram of darkly pigmented yeasts can reach >5 °C warmer than the ambient temperature within minutes of sunlight exposure (13). The identification of heat-producing bacteria (16) suggests that a microbial community can produce enough thermal energy and maintain warmer temperatures than the surroundings. More thermal information of microbial specimens is needed to reveal any potential thermal patterns between fungal genera, species, and lifestyles.

The mushroom coldness was observed during the whole *P. ostreatus* fruiting process. The decrease in temperature during fruiting appeared to be proportional to the mushroom size, which is likely related to an increase in mushroom thermal mass or to an unknown age-related structural organization mediating more heat loss. The observation that the mushroom is coldest when still attached to the mycelium is consistent with prior observations (10, 11) and indicates that heat loss is highest when connected to the mycelial network, which provides access to water. This increase in temperature after detachment is also observed in leaves (17). The observation that the fruiting site of mycelium remained relatively cold after mushroom detachment suggests that mushroom heat loss translates to the mycelium level. The thermal images of *P. ostreatus* mushroom also suggest that heat dissipation is more efficient at

certain discrete areas on the mushroom flush. The relatively cold temperatures recorded underneath the mushroom cap make sense considering the relatively high surface area exposed by the gills (8). The different spatiotemporal changes in mushroom temperature during heating and cooling incubations suggests that discrete areas of the mushroom are more efficient at gaining or dissipating heat. Different areas of the mushroom may contain different hyphal structural organizations and/or water content affecting transpiration rates and thermal properties. The change in *P. ostreatus* mushroom average temperature during heating and cooling resembles the phenomena of thermal hysteresis, a process where previous heat dissipation events influence subsequent heat exchanges and temperature changes.

Our data shows that fungal hypothermia is mediated via the evaporation of fungal-associated water. In plants, transpiration occurs mainly at the leaf level and is regulated via stomas, but any analogous structure in mushrooms has not been identified. Our data with light and dark *A. bisporus* mushroom pilei confirm that evaporative cooling accounts for mushroom coldness. Both light and dark mushroom pilei exhibited similar temperature changes, which suggest that pigmentation has an effect too close to our limits of thermal detection or no effect on heat dissipation in mushroom pilei. The high-water content of mushrooms is consistent with previous reports (18) and explains their high transpiration capacity (10). Their high-water content implies that mushroom's thermal properties must be close to those of liquid water. Other fruits are also highly hydrated (i.e., cucumber); however, it is unknown how their transpiration rate compares to those of mushrooms.

Our data on yeast and molds biofilms also suggest that evaporative cooling accounts for their relatively cold temperatures. The condensed water on the plastic cover above yeast and molds biofilms provides evidence for evaporative cooling. The difference in water content between the encapsulated and acapsular mutant colonies of *Cryptococcus* can be explained by the capsule, which is mostly water (19). Although the acapsular mutant of *C. neoformans* contains ~10% less water mass, it shows more water condensation and colder temperatures relative to the encapsulated strain. The data suggest that the capsule can retain water from evaporating, which would be consistent with the proposed role of microbial capsules in preventing desiccation in the environment (20). The observed differences between a normal and a mutant of *Cryptococcus* biofilms also suggest that water condensation and biofilm temperature may serve as proxies in genome-wide genetic screens for the identification of molecular mechanisms of thermoregulation in yeast.

What is the biological advantage of fungal hypothermia? Mushrooms are considered the reproductive organ of mycelium, and their relatively cold temperature are proposed to be important in spore release (10, 11). Spore discharge is trigger by the mass and momentum transfer of microscopic drops of fluids on the spore surface (aka Buller's drop) (21). Buller's drop is formed by the condensation of water from the moist air (22). The increased surface area by the gills is believed to enhance the airflow and water condensation, further favoring spore detachment (10). In addition to spore discharged, cold temperatures could have a more fundamental role in fungal sporogenesis. There are many examples in

nature were sporogenesis is associated with cold temperatures (i.e., human spermatogenesis). Fungal hypothermia may be a biological advantage related to DNA recombination fidelity.

Understanding the mechanisms of fungal thermoregulation is important for the development and optimization of novel biotechnologies and biomaterials. Our data shows that the relatively high transpiration rate of mushrooms could be exploited to develop a natural air-conditioning device. Our data is consistent with previous reports (10) and suggests that mushrooms can be used to develop a passive air-cooling system. Mushroom-based air cooling depended on the relative humidity, and for detached *A. bisporus* mushroom pilei, evaporative cooling is compromised at relative humidity close to 60%. Subsequently, the transpiration rate of mushrooms can be used to humidify the surrounding air. Better results could be achieved using mushroom species with higher transpiration rates, still attached to their mycelium, and on a device that regulates the accumulation of moisture. Mushrooms can be used not only to cool the surrounding air but also to humidify and even purify it without electricity and CO2 emissions. These findings suggest the possibility of using massive myco-cultures for cooling selected environmental areas and even the planet. For example, extensive myco-culture in soils shaded by forests could reduce the temperatures of these locales that could mitigate global warming trends, at least locally. Given that fungi live on soils and comprise 2% of the earth biomass (3), that fungi are 2-4 °C cooler than their environment, that the average surface temperature of the earth is ~14 °C (23), and assuming linearity in heating and cooling, we estimate that without fungi the temperature of the planet would be 0.25-0.5% warmer.

In conclusion, this study reveals the cold nature of fungal organisms and evaporative cooling as a fundamental mechanism for heat loss and thermoregulation for this kingdom. Fungal hypothermia implies that their heat loss is much greater than the production of heat via metabolism. Their relative cold temperatures also imply that the flow of surrounding thermal energy will move towards the fungus. The high-water content and transpiration rate of fungi implies that their molecular composition and structure enables the efficient transfer of thermal energy and water. Infrared imaging enables the study of mushrooms, molds, and yeasts as novel model systems to study thermal biology and thermodynamics at the community level. A yeast model to study thermal biology is interesting as it could allow the screening of genetic and epigenetic mechanisms regulating thermodynamics and thermal fitness. Understanding how fungal organisms dissipate heat can inspire novel biotechnologies for air-conditioning and the building of infrastructures.

Materials and Methods

All wild mushrooms specimens were obtained from Lake Roland Park in the State of Maryland during the evenings of July 5, 6, and 9th of 2019. Partial and non-official identification of specimens was made based on a visual inspection and photograph analysis via crowdsourcing the Internet. *Candida* spp., *Clodosporium* spp*.,* and *Penicillium* spp. were obtained from mosquito gut isolates by the Dimopoulos Laboratory at the MMI Dept. *Rhodotorula mucilaginosa* was isolated from a contaminated YPD agar plate in our laboratory. *C. neoformans* Serotype A strain H99 (ATCC 208821), acapsular cap59 mutant yeasts and *Penicillium* spp. molds were grown in Sabouroaud Dextrose agar or liquid media for 3-5 days at 30 °C and 24 °C, respectively. *Pleurotus ostreatus* was purchase from The Mushroomworks (Baltimore, MD) as an already-inoculated substrate contained in a 6-pound clear filter patch bag. Fruiting was triggered by making a single 4-inch side cut on the bag and let standstill at 24 °C for seven days. Mushroom flush was detached from mycelium on day four after it started fruiting. Light and dark *Agaricus bisporus* were purchased from New Moon Mushrooms (Mother Earth, LLC., Landenberg, PA, USA) and L. Pizzini & Son, Inc. (Landenberg, PA, USA), respectively.

Thermography. Wild mushroom temperatures were measured using a FLIR C2 IR camera (FLIR Systems, Wilsonville, OR). The camera specifications are 80x60-pixel thermal resolution; 640x480-pixel visual camera resolution; 7.5-14 µm spectral range of camera detector; object temperature range of -10 to 150 °C, accuracy ±2 °C or 2%, whichever is greater, at 25 °C nominal; thermal sensitivity: <0.10 °C; adjusted emissivity to 0.96. The ambient temperature was derived from a card-containing black vinyl electrical tape with an emissivity of 0.96 and aluminum foil (emissivity 0.03). The black tape and aluminum foil were included in the picture as a reference for ambient and reflective temperature readings, respectively (**Fig. S6 a**). How effective is the black vinyl tape to reproduce ambient temperatures? This was tested by thermal imaging of the reference card following ~20 minutes incubation inside three temperature-controlled rooms, set to approximately 5, 25, and 38 °C. The temperature readings obtained from the black tape using the thermal camera were 5.2 (5.2/5.4 min/max), 25.5 (25.5/25.5 min/max), and 37.9 (37.7/38.1 min/max) °C, respectively; demonstrating that black tape radiative temperature corresponded to the ambient temperature. The temperature readings between the thermal camera and a mercury thermometer matched clearly (**Fig. S6 b**), confirming that black tape radiative temperature matched the temperature of rooms, hence serving as a useful reference for ambient temperature.

The thermography of yeast and molds colonies/biofilms was done similar to was described previously (13). Thermal images of yeast, molds, and commercial mushrooms (*P. ostreatus* and *A. bisporus*) were taken inside a white Styrofoam box (30 x 27 x 30 mm, and 3.5 mm wall thickness) to prevent heat loss and radiation noise from surroundings. Prior to imaging yeasts and mold specimens, the sample plates were incubated at 45 or 37 °C. This incubation was required to detect a temperature difference between the colony and the agar as dictated by our thermal camera resolution. Following 1-hour incubation period, the yeast/mold containing plates were immediately transferred inside a Styrofoam box. Next, the box was closed with a lid having a hole fitted to a FLIR C2 IR camera (FLIR Systems,

Wilsonville, OR). The camera detector is set at 2.5 nm distance from the specimen. The temperature of the *P. ostreatus* mushroom flush was monitored during heating and cooling by placing the mushroom inside a warm room (37 °C, <10% RH) or cold room (4 °C, ~30% RH) for 137 and 104 minutes, respectively. Thermal images of mushroom flush were taken inside the Styrofoam box at different time intervals. All apparent temperatures of yeasts, molds, and mushrooms were obtained from infrared images using the FLIR Tool analysis software Version 5.13.17214.2001. Plot profiles of thermal images were obtained using the ImageJ software.

Water condensation of fungal biofilms. *C. neoformans* yeast biofilms were prepared by spotting 25 µL of a liquid 2-day old pre-culture onto Sabouroaud agar medium. The liquid pre-cultures were inoculated from a frozen stock and grown for two days at 30 °C (shaking at 180 rpm). *Penicillium* spp. biofilm is naturally formed by inoculating on a Sabouroaud agar plate. Yeast and mold-inoculated plates were grown upright at 24 °C for 1-2 weeks or until water condensation on the lids became visible. The amount of condensed water at the lid above a mold's biofilm or plain agar was collected using a Steriflip® filter vacuum unit (Millipore Sigma) connected to two 50 mL conical tubes; one at each end. Suction was achieved by connecting a small tubing across the filter into one of the conical tubes. A pipet tip connected at the end of tubing facilitated the aspiration of condensed water droplets on the lid and its collection into one of the 50 mL conical for weighting. The water mass was normalized by the condensation area on the lid, which was estimated from digital images using the ImageJ software.

Mushroom dehydration. Mushrooms were dehydrated for five days using a freeze-drying system (Labcono, Kansas City, MO).

Thermocouple-thermometry of pilei. To monitor the temperature of *A. bisporus* mushrooms as a function of time, mushroom pilei of equal masses, kept at 24 °C, were placed on glass trays inside ziplock clear bags (one mushroom per bag). One bag contained 40 grams of desiccating-anhydrous indicating Drierite (W.A. Hammond Drierite Company, LTD), and a second contained 40 grams of distilled water. Thermocouple detectors (K-type) were submerged inside each mushroom cap (centered from top). Bags were then closed and placed inside a warm room (37 °C, <10 % RH), and temperature readings were recorded every second using the Amprobe TMD-56 thermometer (0.05% accuracy) connected to a computer. Each mushroom sample was measured individually inside the warm room.

A mushroom-based cooling device. A prototype for a mushroom-based air-cooling device or MycoCooler™ is showed in (**Figure 4a**). The prototype device was made using a Styrofoam box with dimensions 20 x 21 x 21 cm or a total volume of 8820 cm^3. An inlet aperture of 1-cm diameter and an outlet aperture of 2-cm diameter at opposite ends of the box allowed air flow inside and outside the box containing mushrooms (**Fig S5a**). An exhaust fan (Noctua NF-P12) was glued outside the box on top of the outlet aperture to facilitate the circulation of air (air flow rate of approximately in and out the MycoCooler™ (**Fig S5b**). Approximately, 420 grams of fresh *A. bisporus* mushrooms were placed inside the MycoCooler™ box, which was then closed, and placed inside a larger Styrofoam box with dimensions (30.48 x 30.48 x 30.48 cm or a total volume of 28.32 L (28,316.85 cm^3). This larger box was maintained

inside a warm room (37 °C, <10 % RH) throughout the experiment. The MycoCooler™ containing mushrooms was enclosed inside the larger box once the temperature and humidity values reached steady state. The temperature and relative humidity inside the larger Styrofoam box (**Figure S5a**) were recorded every minute using an Elitech GSP-6 data logger having a temperature accuracy of ±0.5 °C (-20~40 °C) and humidity range 10%~90% and an accuracy of ±3% RH (25 °C, 20%~90% RH).

Quantification and statistical analysis. Details for each statistical analysis, precision measures, the exact value of n (and what n represents; sample size and the number of replicates) for all shown data can be found in the figure legends. We used an alpha level of 0.05 for all statistical tests.

To calculate the mushroom's cooling capacity, we used the MycoCooler temperature change data to estimate the cooling capacity of 420 g of *A. bisporus* mushroom pilei. Cooling capacity was calculated using the energy equation for heat transfer $Q = m \times Cp \times \Delta T$, were m is the mass flow rate of air in kg/s, Cp is specific heat capacity of air in kJ/kg*K, and delta ΔT is the temperature difference in Kelvin. The mass flow rate of air was obtained by multiplying the density of air at 37 °C (1.006 kJ/kg*K) by the fan flow rate, 0.026 m^3/s (taken from equipment specifications). This results in a mass flow rate of 0.02 kg/s, that if multiplied by the heat capacity nominal value of air at 305.15 K (1.006 kJ/kg*K) and the air temperature difference of the enclosed system before and 45 minutes after the addition of mushrooms (37 °C +273.15) – (27 °C +273.15=10 K). This yields a heat transfer or cooling capacity of ~ 20 Watts or 68 British thermal units per hour (BTU/h). This divided by the mass of mushrooms 0.42 kg yields ~68 Watts/kg.

To estimate the temperature of Earth without fungi, we considered the average temperature difference of wild mushrooms, which ranged from ~2-5 °C, and multiply it by the estimated amount of fungal biomass, ~2% (3), such that the temperature associated to the fungi would be ~0.04 - 0.1 °C. We then estimated the global mean surface temperature without the fungal biomass, X=15 °C + (0.04 or 0.1 °C), such that global temperatures would be ~15.04 °C or ~15.1 °C, or ~0.3-0.7 % warmer.

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Figures and Tables

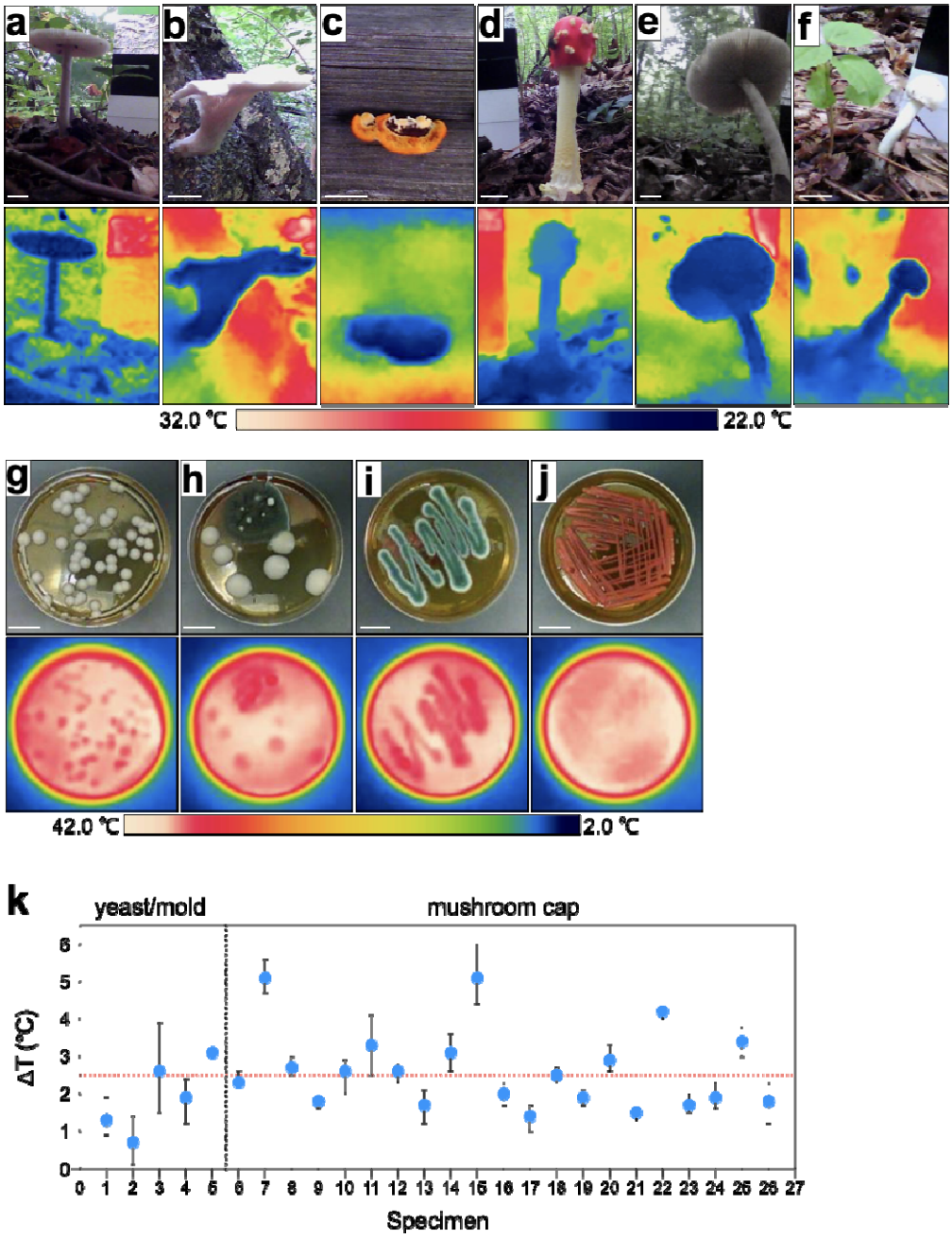

Figure 1. Yeasts, molds, and mushrooms are colder than their environment. thermographic examples of wild mushrooms imaged in their natural habitat: (**a**) *Amanita* spp., (**b**) *Pleurotus ostreatus*, (**c**) *Pycnoporus* spp., (**d**) *Amanita muscaria* (**e**) *Amanita brunnescens,* (**f**) *Russula* spp. (**g**) yeast *Candida* spp. (also seen in **b** as white colonies), (**h**) mold *Cladosporium sphaerospermum* (dark colony), (**i**) mold *Penicillium* spp., and(**j**) yeast *Rhodotorula mucilaginosa*. (**k**) The temperature difference between the surrounding/ambient and fungal specimen. Error bars represent standard deviation. The temperature values of all fungal specimens and surroundings are listed in **Table S1**.

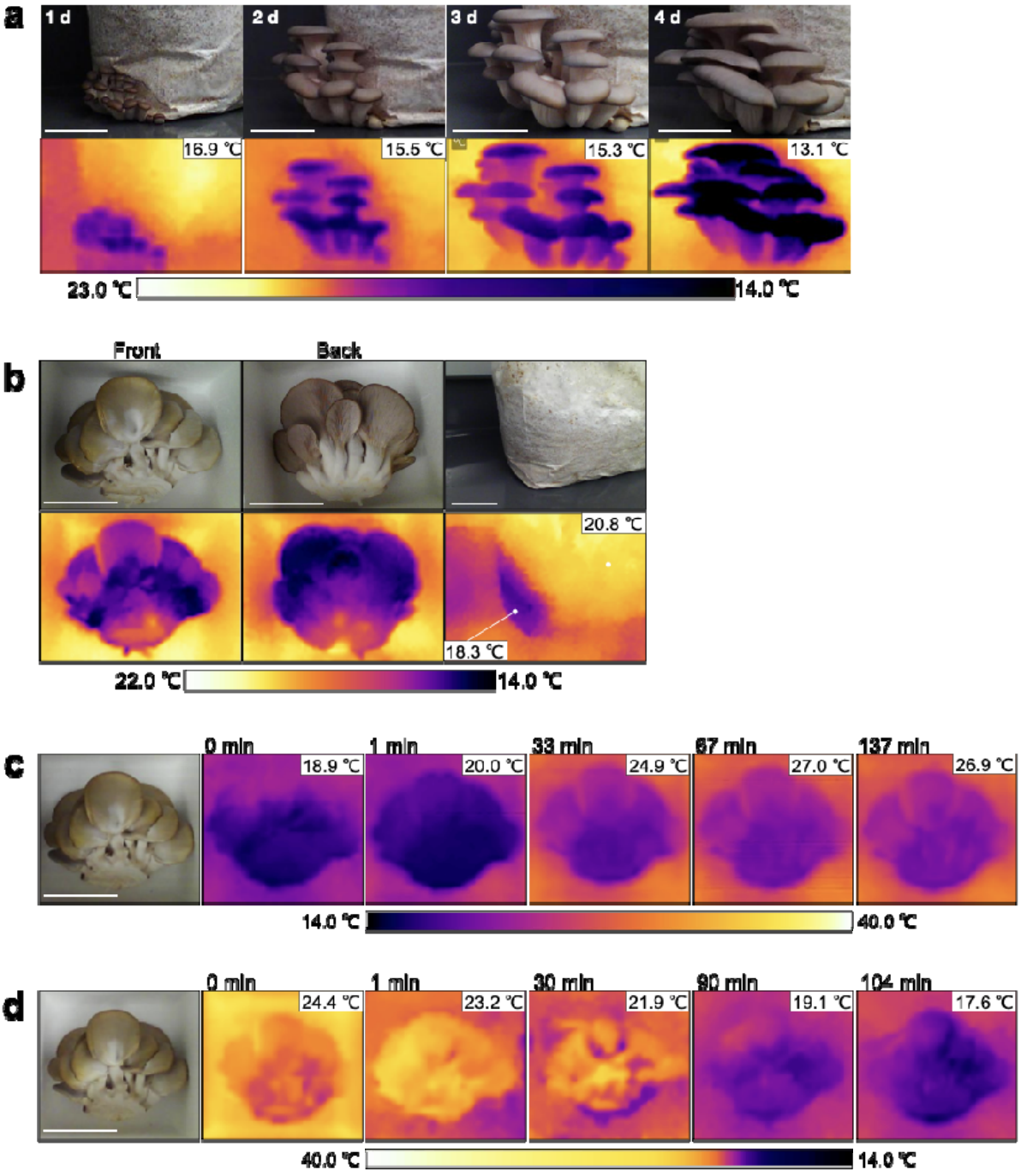

Figure 2. The mushroom temperature during fruiting and during heating and cooling.

(**a**) Visible and thermal images of *Pleurotus ostreatus* during fruiting at temperature-controlled room (22 ± 5°C, 50% RH). Inset temperature values correspond to the lowest temperature signal registered in the thermograph. (**b**) Frontal and back imaging of mushrooms and mycelium bag after detachment at day 4. Thermal imaging of *P. ostreatus* following incubation inside (**c**) warm room at 37 °C, <10% RH followed by incubation inside (**d**) cold room at 4 °C, ~30% RH. Inset temperature values correspond to the lowest and highest temperature signal in the thermographs in (c) and (d), respectively.

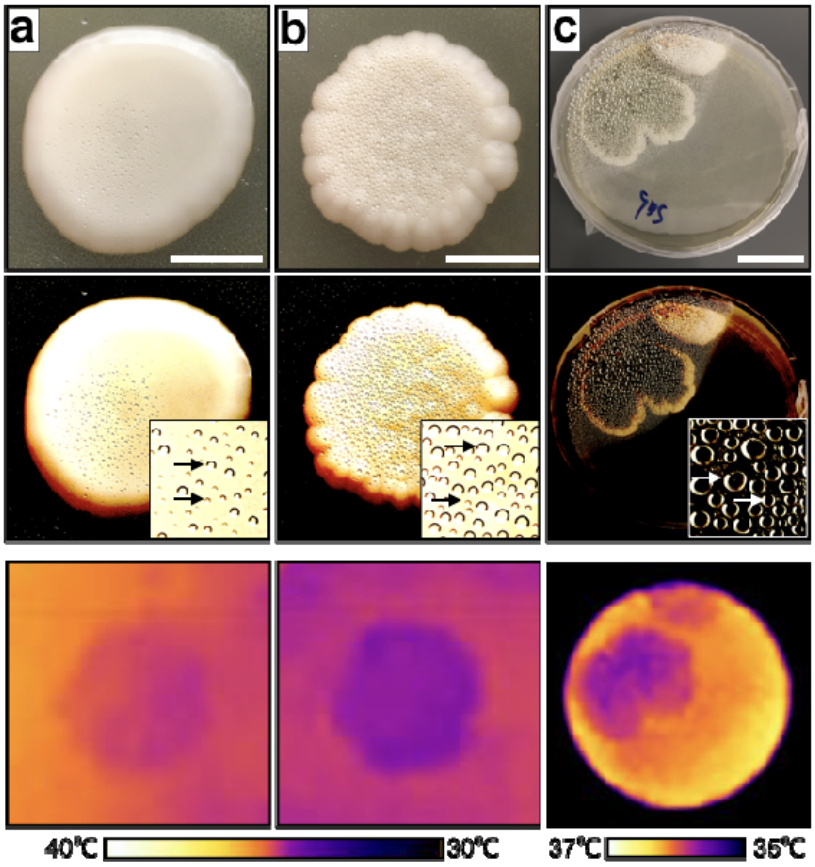

Figure 3. Evaporative cooling in yeast and mold biofilms.

Evidence for evaporative cooling is observed from the condensed water droplets at the lid of petri dish on top of colony/biofilm. Visible (top and middle) and thermal images (bottom) of (**a**) wildtype H99 *Cryptococcus neoformans;* scale bar 1 cm; (**b**) *Δcap59* acapsular mutant of *C. neoform*ans; scale bar 1 cm, and (**c**) normal *Penicillium* spp.; scale bar 3 cm. Visible images were altered to increase contrast and help visualize water droplets (middle row).

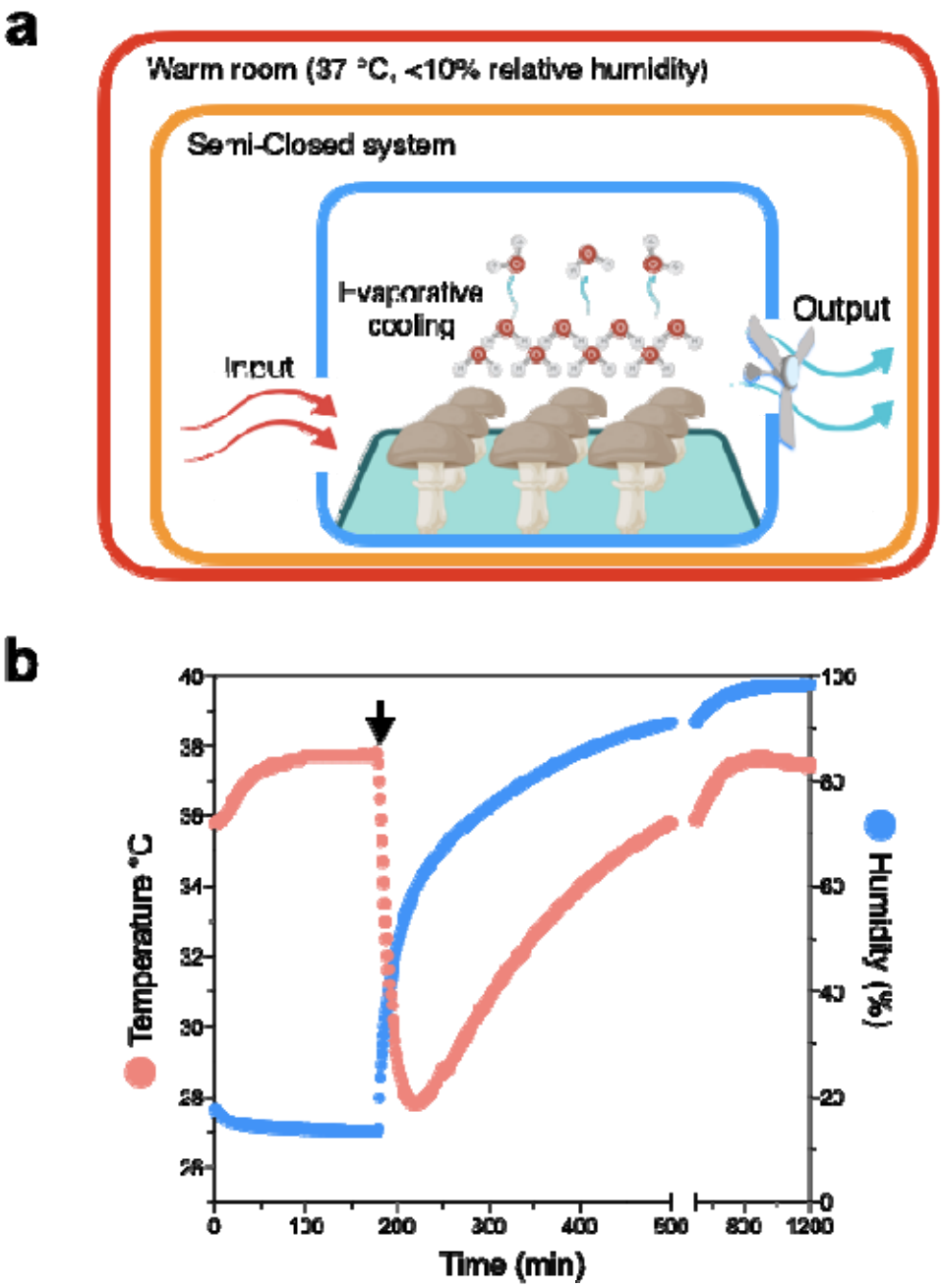

Figure 4. Proof-of-concept of a mushroom-based air conditioning system.

(**a**) Prototype diagram model of MycoCooler™ air conditioning system. Warm air enters an insulated chamber containing mushrooms. As the warm air flows inside the chamber, mushroom-mediated evaporative cooling will cool the air. An exhaust fan will push the cooled air through a HEPA filter to limit spore dispersal and enhance air circulation. The fan can be powered via a photovoltaic cell making this system free of carbon emissions. (**b**) Input and output air temperature and relative humidity as a function of time. A MycoCooler™ prototype was placed inside a semi-closed Styrofoam box. Mushrooms were added once the temperature inside the semi-closed system reached steady-state (black arrow).