

Supporting Information for The hypothermic nature of fungi

Radames J.B. Cordero¹, Ellie Rose Mattoon², Zulymar Ramos^{3,4}, and Arturo Casadevall^{1*}

Arturo Casadevall
Email: acasade1@jhu.edu

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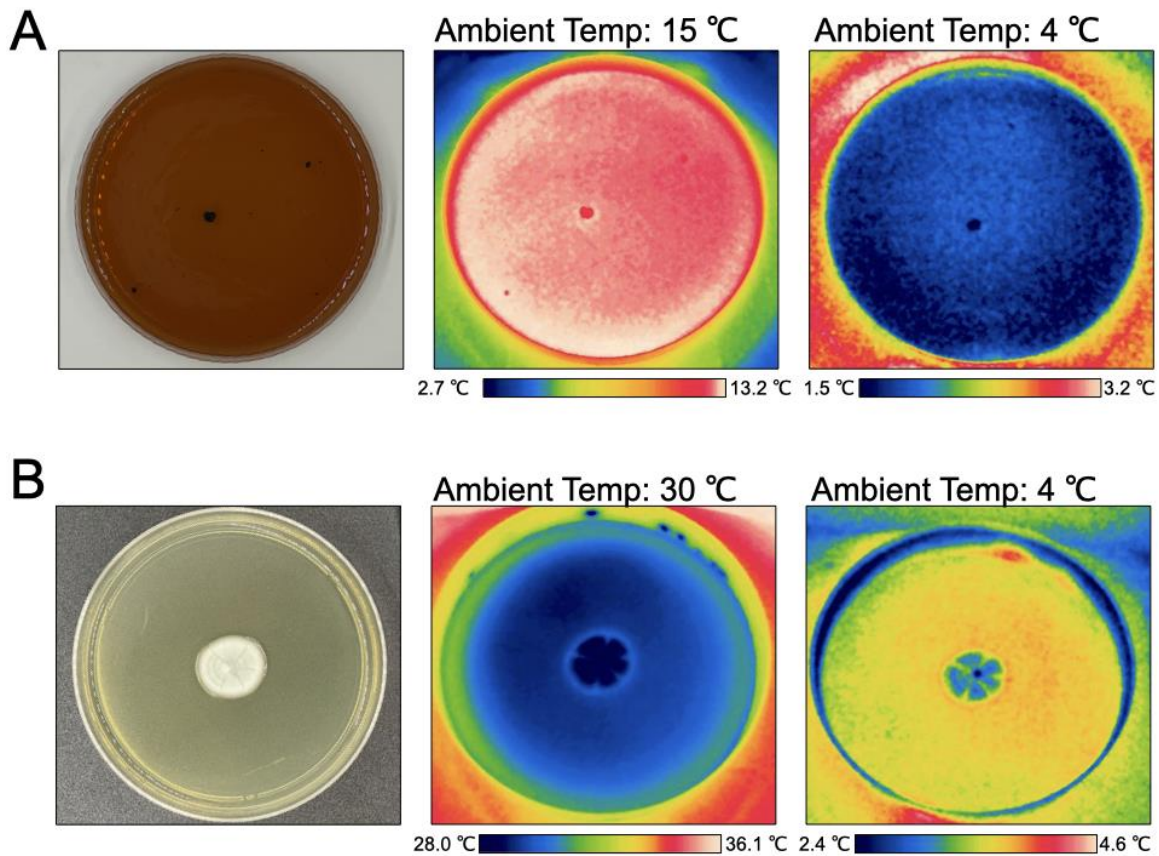


Fig. S2. Fungal colonies remain colder than surrounding agar even under 4 °C ambient temperature. Visible images and IR thermograph representations of (A) a 20-day old colony of *Cryomyces antarcticus* incubated at 15 °C and 4 °C. The average colony temperature of *C. antarcticus* under 15 °C was 11.7 ± 0.6 °C and the temperature of surrounding agar was 12.8 ± 0.4 °C which gives a temperature difference of ~ 1.1 °C. Under 4 °C, the average *C. antarcticus* colony temperature was 1.5 ± 0.1 °C and the surrounding agar was 1.9 ± 0.1 °C which gives a temperature difference of ~ 0.4 °C. (B) A 5-day old colony of *Penicillium* spp., incubated at 30 °C and 4 °C. The average colony temperature of *Penicillium* under 30 °C was 28.7 ± 0.4 °C and the temperature of surrounding agar was 29.4 ± 0.2 °C, which gives a temperature difference of ~ 0.7 °C. Under 4 °C, the average *Penicillium* colony temperature was 1.5 ± 0.2 °C and the surrounding agar was 1.9 ± 0.1 °C, which gives a temperature difference of ~ 0.4 °C.

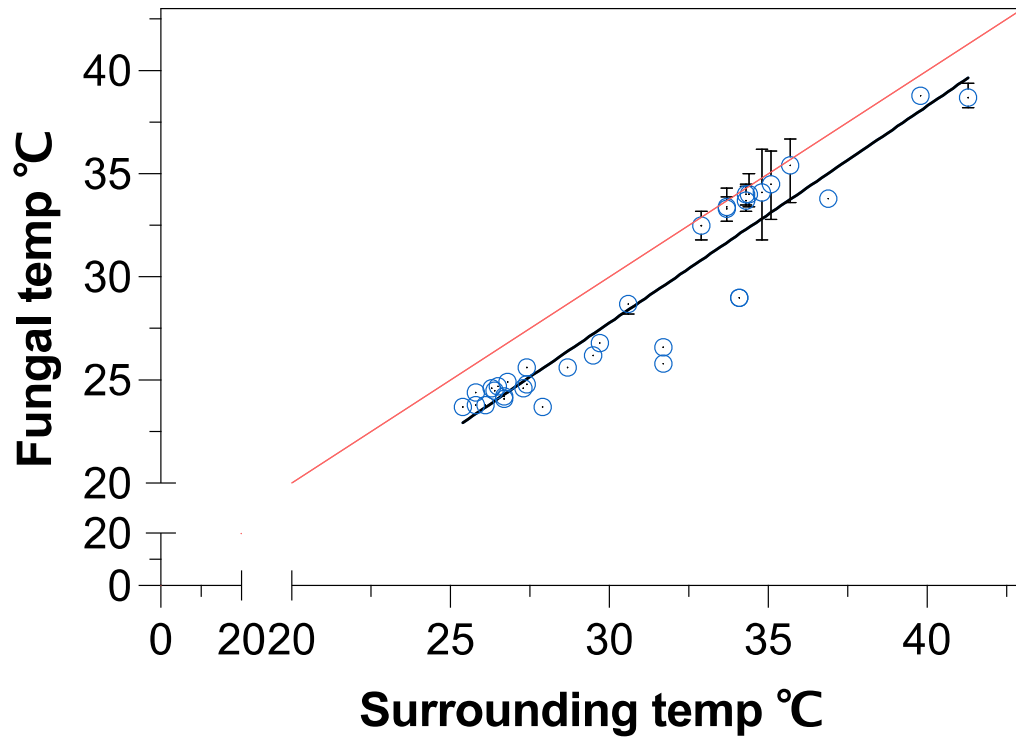


Fig. S3. Linear regression analysis of fungal temperatures as a function of the surrounding temperature. Number of specimens = 34, slope=1.1, $R^2 = 0.9$. Error bars represent the standard deviation from the mean. The red line shows the line of identity.

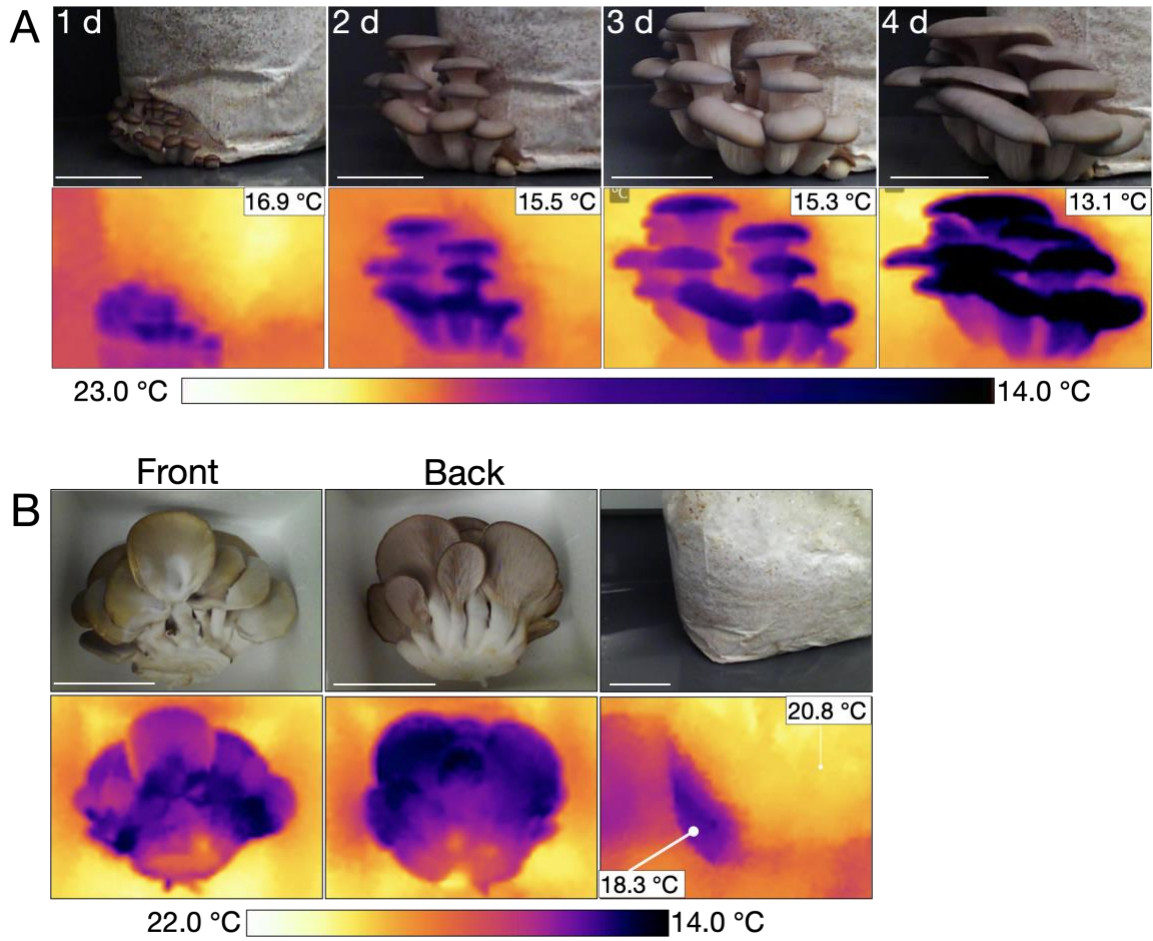


Fig. S4. Temperature of *Pleurotus ostreatus* during fruiting. (A) Visible images and infrared thermographs of *Pleurotus ostreatus* during fruiting while still attached to its substrate in a temperature-controlled room at $22 \pm 5^\circ\text{C}$, 50% RH. Inset temperature values correspond to the lowest mushroom temperature signal registered in the thermograph. (B) Frontal and back imaging of mushroom flush and mycelium-colonized bag after detachment on day 4.

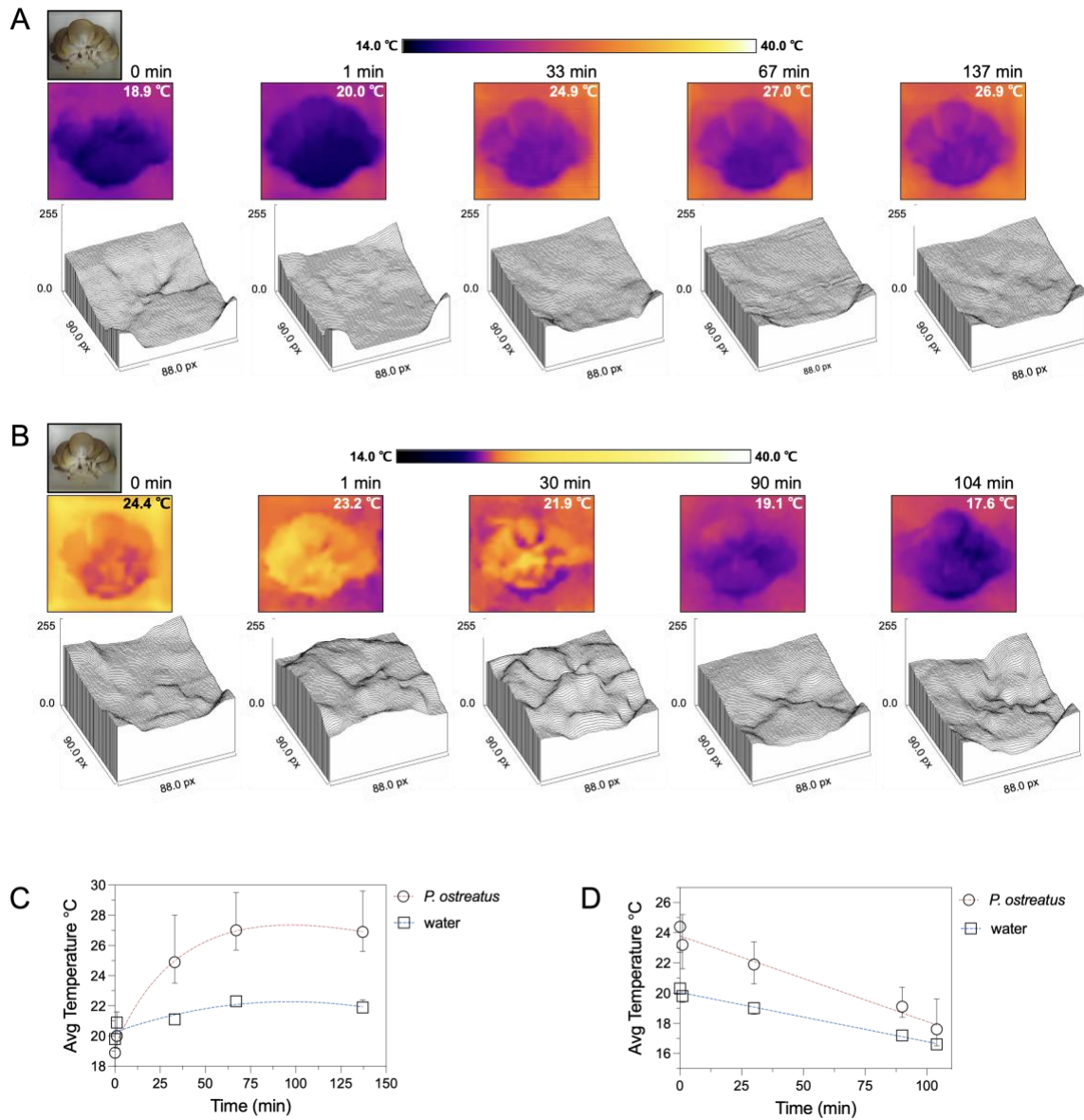


Fig. S5. Changes in *P. ostreatus* mushroom temperature during heating and cooling. Thermal images and plotted profiles of detached *P. ostreatus* mushrooms flush immediately incubated inside a warm room at 37°C and < 10% RH) (A) and subsequently incubated in a cold room at 4°C and ~30% RH (B). Inset temperature values correspond to the lowest and highest temperature signal detected in the thermographs. Mean mushroom temperature as a function of time during heating at 37°C and cooling at 4°C (C and D, respectively). Error bars represent the standard deviation from the mean.

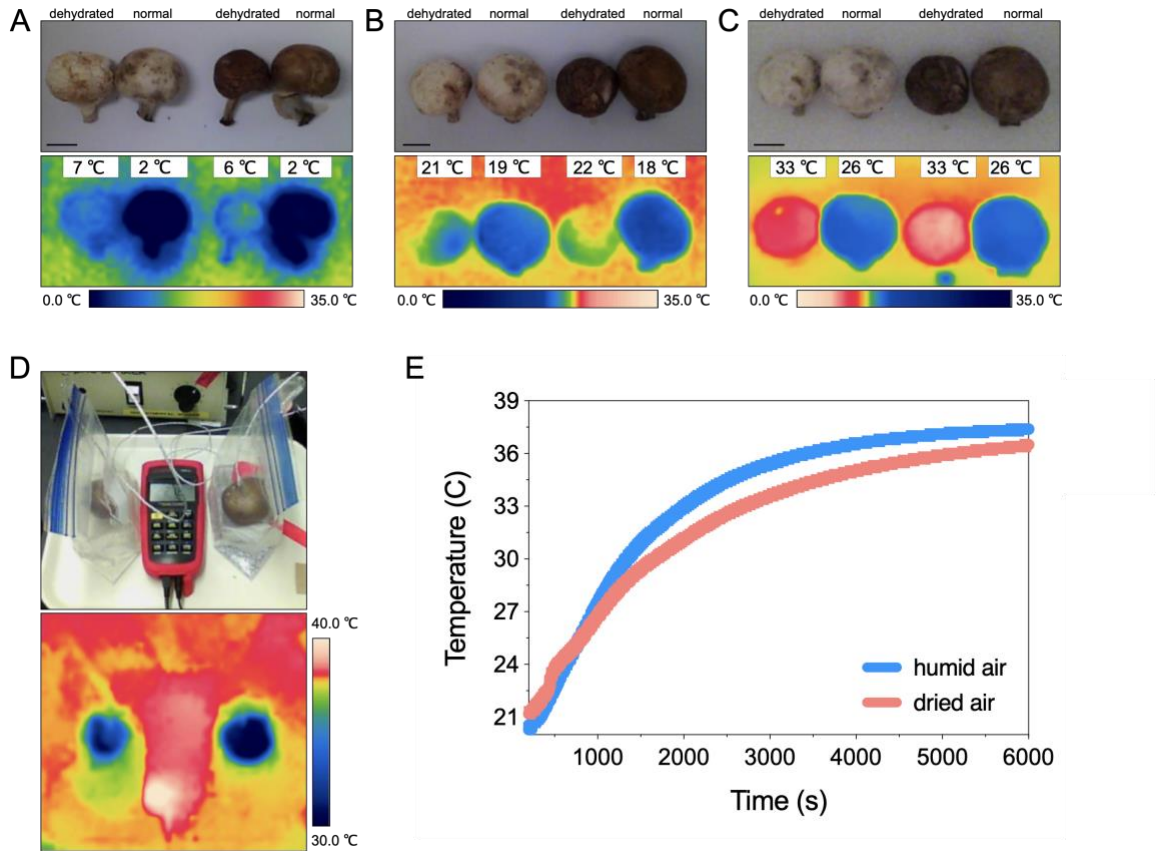


Fig. S6. Mushroom coldness is mediated via evaporative cooling. Dehydrated (*left*) and normal (*right*) versions of light and dark *Agaricus bisporus* detached mushrooms were incubated at (A) 4, (B) 23, and (C) 37 °C ambient temperatures. Normal mushrooms can maintain cooler temperatures regardless of the ambient temperature. (D) Experimental setup to monitor mushroom temperature change as a function of humidity. (E) Mushroom temperature as a function of time was recorded inside enclosed plastic bags containing water (*humid air*) or dessicating Drierite (*dried air*).

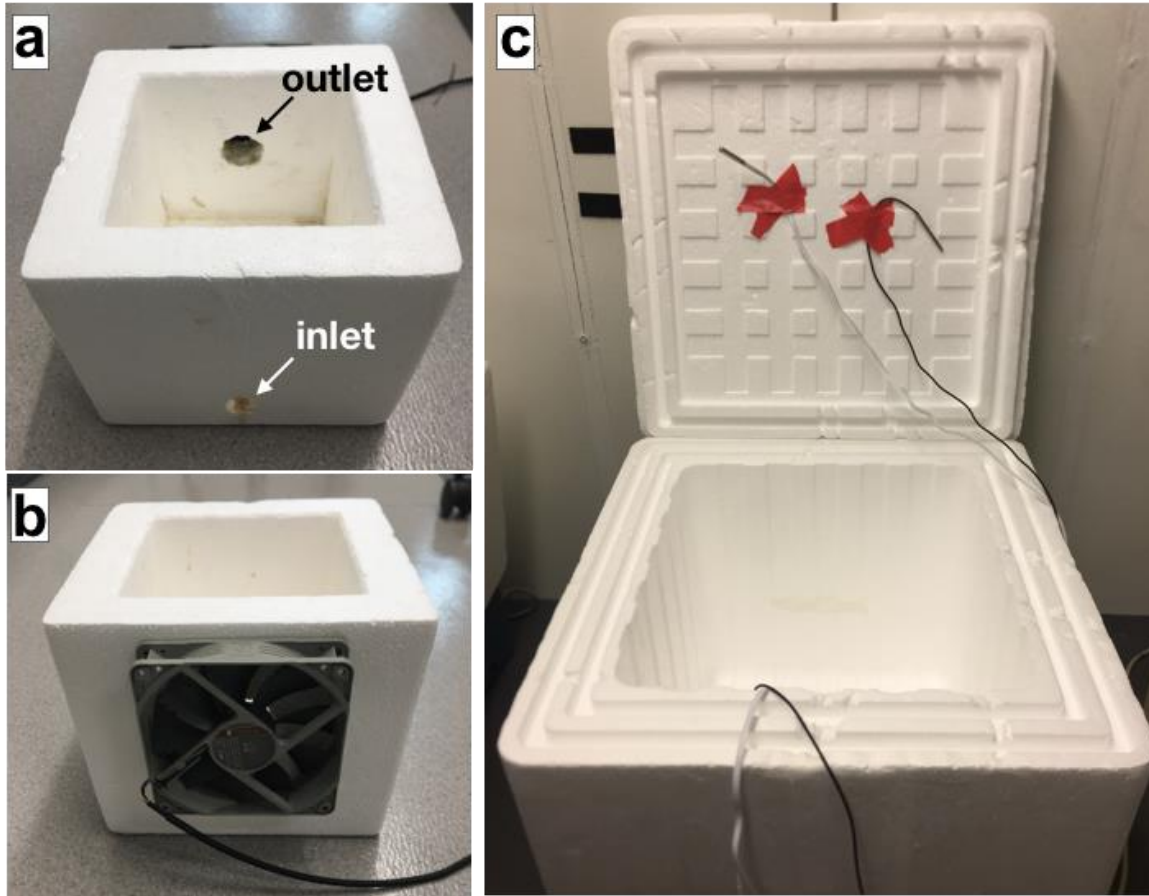


Figure S7. MycoCooler™ prototype. (A-B) The MycoCooler™ prototype is made of a smaller Styrofoam box (lid not shown) with an inlet aperture of 1 cm diameter and outlet aperture of 2 cm diameter. An exhaust fan was glued outside the box centered on top of the outlet aperture. (C) Large Styrofoam box ((30.48 x 30.48 x 30.48 cm) having an area of 28,317 cm³. Temperature and humidity probes (white and black cables, respectively) tapped into the inner side of the lid can be observed.

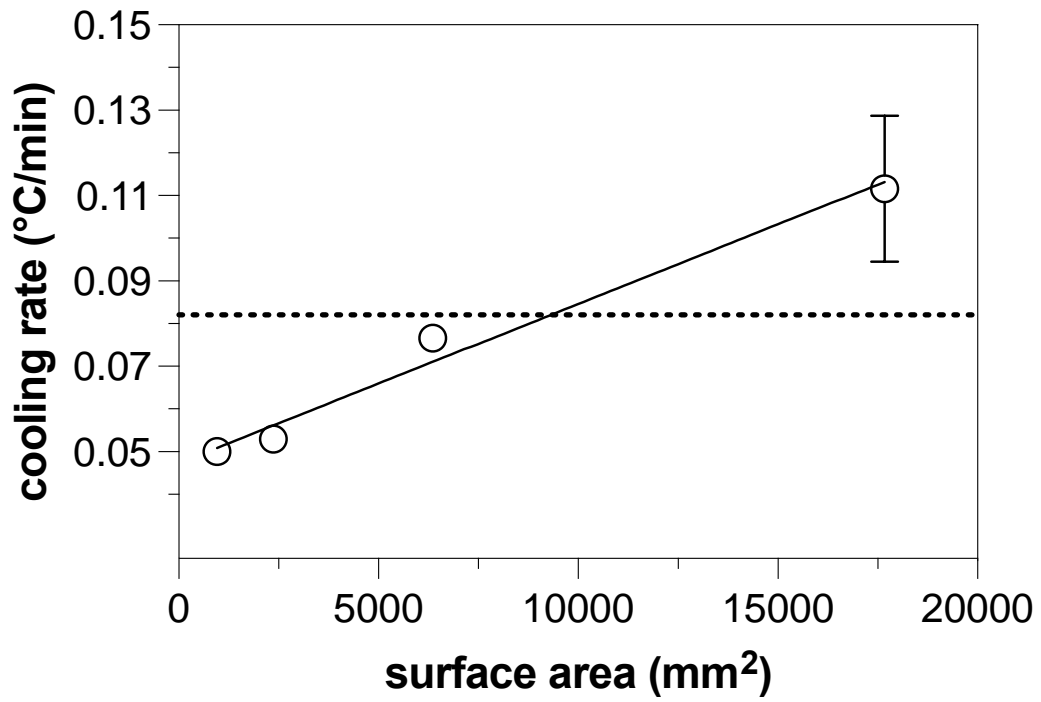


Fig. S8. Cooling rate of water as a function of surface area. Error bars correspond to the standard deviation from the mean (n=3 replica). Horizontal lines mark the mean cooling rate of single *Agaricus bisporus* mushrooms with equivalent mass (0.082 ± 0.01 °C/min, n=3).

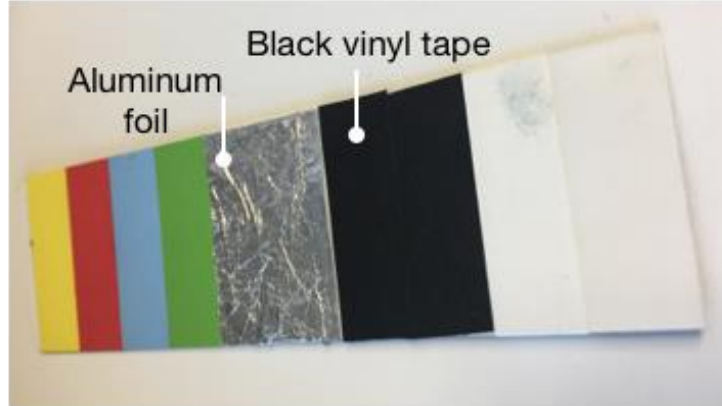
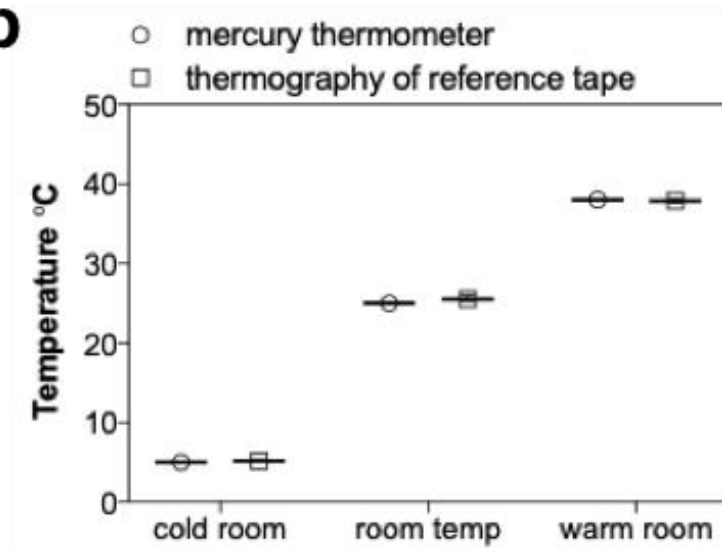
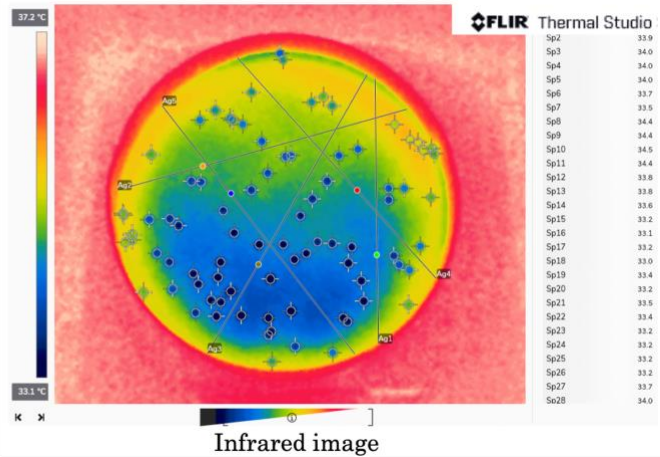
a**b**

Fig S9. Black vinyl tape as reference for ambient temperature. (A) Reference card containing black vinyl tape and aluminum foil. (B) Temperatures measurements inside cold (5 °C), warm (37 °C), and regular (25 °C) rooms using a mercury thermometer and thermography of reference card-black tape.



Visible image



Infrared image

Figure S10. Example of temperature measurements of single yeast colonies and surrounding agar using the FLIR Thermal studio software.

Table S1. The surface temperature (°C) of wild mushrooms using infrared thermography.

Fungal Specimen	Specimen Temperature				Surrounding Agar Temperature*				Temperature Difference**
	n	mean	max	min	n	mean	max	min	
<i>Amanita</i> spp.	1	23.8 (23.9)	24.1 (23.8)	23.7 (24.1)	1	26.1	26.2	26.1	2.3
<i>Pleurotus ostreatus</i>	1	26.6	26.7	26.6	1	31.7	31.9	31.3	5.1
<i>Amanita muscaria</i>	1	24.6 (24.4)	24.7 (24.6)	24.4 (24.1)	1	27.3	27.4	27.2	2.7
<i>Amanita brunnescens</i>	1	24.7 (24.9)	24.8 (25.0)	24.7 (24.8)	1	26.5	26.6	26.4	1.8
<i>Russula</i> spp.	1	24.8 (24.9)	25.2 (25.1)	24.7 (24.8)	1	27.4	27.6	27.0	2.6
<i>Boletus separans</i>	1	26.2	26.5	25.9	1	29.5	30.0	29.1	3.3
<i>Russula</i> spp.	1	24.1 (24.5)	24.3 (24.5)	24.0 (24.4)	1	26.7	26.8	26.5	2.6
<i>Amanita</i> spp.	1	24.6 (24.5)	24.8 (24.7)	24.5 (24.3)	1	26.3	26.6	26.2	1.7
<i>Thelephora</i> spp.	1	25.6	25.9	25.3	1	28.7	28.9	28.3	3.1
<i>Cerrena unicolor</i>	1	29.0	29.2	28.6	1	34.1	34.6	33.2	5.1
<i>Cantharellus</i> spp.	1	23.8	23.9	23.7	1	25.8	26.0	25.7	2.0
<i>Russula</i> spp.	1	24.4 (24.2)	24.7 (24.2)	24.2 (24.1)	1	25.8	25.9	25.7	1.4
<i>Hortiboletus</i> spp.	1	24.2 (24.0)	24.3 (24.0)	24.1 (24.0)	1	26.7	26.8	26.5	2.5
<i>Marasmius capillaris</i>	1	24.5	24.6	24.4	1	26.4	26.5	26.3	1.9
<i>Coprinellus micaceus</i>	1	26.8	27.0	26.5	1	29.7	29.8	29.6	2.9
undetermined	1	23.7 (23.9)	23.8 (24.1)	23.5 (23.7)	1	25.4	25.5	25.3	1.7
undetermined	1	24.9	25.0	24.7	1	26.8	27.0	26.5	1.9
undetermined	1	25.6	25.7	25.6	1	27.4	27.9	27.1	1.8
undetermined	1	29.0	29.2	28.6	1	34.1	34.6	33.2	5.1
<i>Pleurotus ostreatus</i>	1	25.8	26.0	25.7	1	31.7	31.9	31.3	5.9

* For wild mushroom specimens, the surrounding temperature corresponds to the ambient air temperature. The stalk (or stem) temperature of some specimens is shown in parenthesis but not used to calculate the temperature difference between fungal specimens and surroundings.

Table S2. The temperature (°C) of yeast and mold colonies and surrounding agar using infrared thermography.

Fungal Specimen	Incubating Temperature	Specimen Temperature				Surrounding Agar Temperature				Temperature Difference*
		n	mean	max	min	n	mean	max	min	
<i>C. neoformans</i> H99	37	1064	35.4±0.8	36.7	33.6	55	35.7±0.7	36.7	34.3	0.3±0.1
<i>C. neoformans</i> cap59		872	34.1±1.0	36.2	31.8	60	34.8±0.8	36.2	33.4	0.7±0.1
<i>C. neoformans</i> B3501		9	33.7±0.4	34.4	33.2	45	34.3±0.5	35.3	33.6	0.6±0.2
<i>E. dermatitidis</i>		400	34.0±0.3	35.0	33.4	60	34.4±0.5	35.7	33.6	0.4±0.1
<i>C. albicans</i>		669	34.5±0.8	36.0	32.8	60	35.1±0.7	36.0	34.0	0.6±0.1
<i>C. tropicalis</i>		105	33.4±0.7	34.3	32.7	45	33.7±0.7	35.7	32.8	0.3±0.1
<i>C. glabrata</i>		213	33.3±0.2	33.9	32.9	42	33.7±0.3	34.6	33.1	0.4±0.1
<i>C. auris</i>		298	34.0±0.2	34.5	33.5	45	34.3±0.3	35.0	33.7	0.3±0.1
<i>C. krusei</i>		18	32.5±0.5	33.2	31.8	41	32.9±0.6	34.0	31.8	0.4±0.2
<i>C. neoformans</i> B3501	30	80	28.8±0.3	29.5	28.1	53	29.4±0.3	30.2	28.8	0.6±0.1
<i>C. haemulonii</i>		86	29.3±0.3	29.8	28.7	52	29.7±0.4	30.1	29.0	0.4±0.1
<i>C. duobushaemulonii</i>		86	29.1±0.3	29.6	28.2	33	29.6±0.4	30.2	29.0	0.5±0.1
<i>S. cerevisiae</i>		93	28.8±0.3	29.2	28.3	27	29.1±0.2	29.5	28.7	0.3±0.1
<i>R. mucilaginosa</i>		115	28.4±1.7	31.5	26.4	51	29.3±1.6	31.5	26.8	0.9±0.3
<i>G. simplex</i>		27	29.8±0.1	30.1	29.6	30	30.6±0.1	30.8	30.8	0.8±0.0
<i>C. sphaerospermum</i>		6	29.4±1.1	29.8	28.8	6	30.0±1.2	30.3	30.1	0.6±0.1
<i>Penicillium</i> spp.		33	29.6±0.6	29.8	29.4	33	30.4±0.5	30.8	30.4	0.8±0.1
<i>A. niger</i>		15	26.1±1.9	27.4	25.0	17	28.0±2.0	28.2	27.8	1.9±0.7
<i>C. antarcticus</i>	15	48	11.7±0.6	12.7	10.4	38	12.8±0.4	13.6	12.1	1.1±0.1

* Difference between mean surrounding temperature and mean specimen temperature taking into account the standard deviation using the formula: square root ((stdev1²/n1) + (stdev2²/n2)).

Table S3. Average, minimal, and maximal temperatures (°C) of light and dark *A. bisporous* mushroom caps in normal versus dehydrated states following 1 h incubation at 4, 24, and 37 °C.

Ambient Temp	Light mushrooms caps						Dark mushrooms caps					
	Normal			Dehydrated			Normal			Dehydrated		
	avg	max	min	avg	max	min	avg	max	min	avg	max	min
4	1.7	2.2	1.4	6.9	8.0	6.1	1.6	2.7	1.1	6.4	7.2	5.6
24	18.8	20.0	17.9	20.1	21.7	18.9	18.1	19.2	17.6	21.7	22.5	21.0
37	26.3	27.3	24.7	32.9	33.3	32.0	26.4	27.6	24.8	33.3	33.8	31.5

Table S4. Water mass percentage of *A. bisporous* mushrooms and *C. neoformans* yeast colonies. Percent water mass was calculated by the mass difference before and after lyophilization. Values represent different biological replicas.

Specimen	Percent of water (% m/m)
<i>A. bisporous</i> mushrooms	93.6 ± 0.4*
Encapsulated <i>C. neoformans</i>	90.2, 89.7, 89.0, 89.6
Acapsular <i>C. neoformans</i>	81.8, 81.8, 82.3, 81.5
Unidentified mushroom specimens	83.3, 88.1, 89.9, 91.6, 82.1, 80.7, 57.4

*Average and standard deviation from three light and three darkly pigmented specimens.

Table S5. Total water mass condensed on lids divided by colony or agar area.

Sample replica	Water mass per area (mg/m ²)	
	biofilm	agar
1	6.4	0.2
2	10.0	0.8
3	5.0	0.4
4	3.6	0.4