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CHILLING AND FROST TOLERANCES OF A LUMINOUS *MYCENA* SPECIES (AGARICALES, BASIDIOMYCOTA) IN NORTHERN JAPAN

Key words: *cold adaptation, cryophilic, fungi, mycelial growth, psychrophilicity, psychrotolerant, psychrotroph, thermal dependence.*

Mycena is a genus of basidiomycetes and includes several luminous species, especially *M. chlorophos* (MC) [8]. Luminous species of *Mycena*, including those from Japan, are distributed in tropical and subtropical regions. However, Oba and Hosaka [8] also listed eight *Mycena* species in Japan that were potentially luminous, and some were recorded from the snowy region [1—4, 9].

Recently, a luminous unidentified *Mycena* sp. (Yukiguni-yakō-takē in Japanese, meaning snow country luminous mushroom) (LMS) was found in mainland Japan (Honshu), including the snowy region [5, 6], and the phylogenetic analysis suggested that LMS and MC formed a sister group. In this short article, we compared the physiological characteristics of both species and aimed to elucidate the cold adaptation of LMS, thereby discussing the expansion of the ancestor species of MC, which is widely distributed in the subtropics, into cold regions.

Basidiocarps of LMS were collected in Fukaura Town (40.56°N, 139.97°E) and Towada City (40.57°N, 140.98°E) in July 2023. Stipes were removed from basidiocarps. Cut basidiocarps were hung on the inside of the covers of Petri dishes containing potato dextrose agar (PDA; Becton Dickinson and Company, USA) supplemented with streptomycin, using double-sided adhesive tape, and incubated at room temperature for 1 day. Basidiospores that fell on PDA germinated and mated with each other. Mycelia with clamp connections were observed under the microscope and inoculated on new PDA at 20 °C for 2 weeks. MC NBRC 31858 was used for the control of this study.

Mycelial discs of 5 mm in diameter were cut from the margin of an actively growing colony, transferred to the centers of PDA plates, and inoculated on four different agar plates (PDA, oatmeal agar (OA), malt extract agar (MA), Sabouraud agar (SA)), all media were obtained from Becton Dickinson and Company (USA), at 20 °C. Mycelial discs were also inoculated onto PDA at 7 temperatures ranging from 4 to 35 °C, in triplicate. After 1, 2, and 3 weeks of inoculation, the colony diameters were determined. The linear mycelial

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growth rate per week was calculated after the initial lag period.

Tested fungi were cultured on PDA and OA at 10 °C for 1 month. Mycelial discs were frozen at –20 °C in a programmed freezer MPF-100 (EYELA, Japan) at 10 °C/h in a test tube [7]. They were then thawed at 2 °C for 8 h, transferred to fresh PDA plates, and incubated at 20 °C for 3 weeks.

Prepared isolates of LMS and MC NBRC 31858 were cultured on four kinds of agar plates at 20 °C. Mycelial growth of LMS was slower than that of MC. The average mycelial growth rate of LMS from Fukaura was 5.31 (PDA), 9.11 (OA), 12.53 (MA), and 7.93 (SA) mm/week, respectively. From Towada strain, these values were 8.13, 10.41, 10.63, and 10.61 mm/week, and for MC NBRC 31858, they amounted to 13.66, 12.48, 16.0, and 13.99 mm/week.

Optimal temperature of mycelial growth in tested fungi was 25, and maximal temperature of them was <35 °C, respectively (Fig. 1). Tested fungi did not grow at 35 °C. Minimal mycelial growth temperature of MC NBRC 31858 was >4 °C; mycelium did not grow, but they were alive in this condition (Fig. 2). On the other hand, both LMS isolates could grow at 4 °C. These results suggested that LMS and MC were psychrotolerant, as indicated by their thermal dependence on mycelial growth, and that MC could survive the unfrozen winter in Japan but did not grow under these conditions. LMS was better adapted to the unfrozen winter and could grow under these conditions. This small difference in physiological characteristics between MC and LMS may help explain their distribution in Japan.

Mycelia of tested fungi on PDA lost viability after the freeze–thaw treatment (Table). However, a few mycelia on OA had frost resistance. Fungi in permafrost are characterized by both the presence of

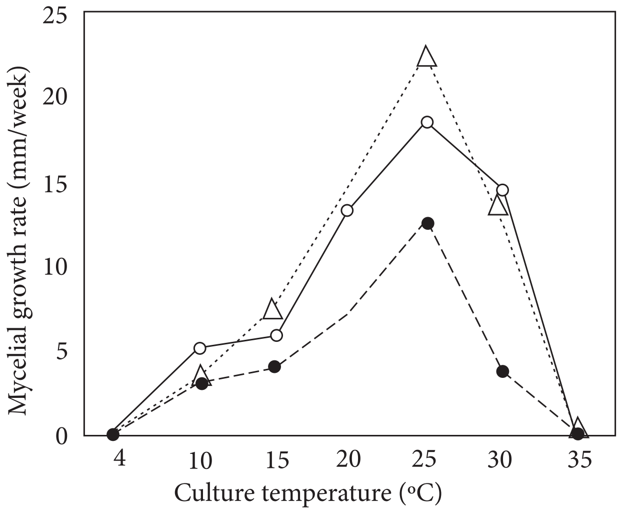

Fig. 1. Thermal dependence of mycelial growth of luminous *Mycena* spp. in Japan. Thermal dependence of mycelial growth between 4 and 35 °C. Open cycles: isolate of luminous *Mycena* sp. from Fukaura Town, Aomori Prefecture, Japan; closed cycles: it from Towada City, Aomori Prefecture, Japan; triangles: *Mycena chlorophos* NBRC 31858

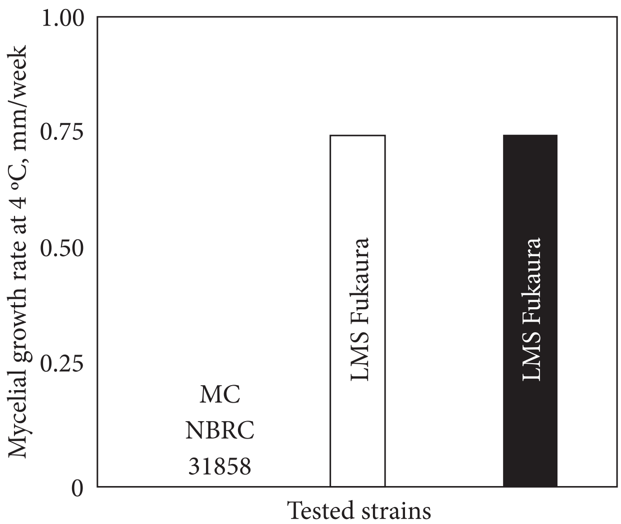

Fig. 2. Mycelial growth of luminous *Mycena* spp. in Japan at 4 °C

Freezing tolerance of luminous *Mycena spp.* in Japan

Experimental conditions	Mycelial survivals (number of regrowth mycelial discs/tested mycelial discs)					
	luminous *Mycena* sp. from				*Mycena chlorophos*	
	Fukaura		Towada		NBRC 31858	
Precultured media	PDA [1]	OA [2]	PDA	OA	PDA	OA
Freezing/thawing cycles						
1	0/5	3/5	0/5	4/5	0/5	2/5
2	NT [3]	3/5	NT	2/5	NT	3/5
3	NT	2/5	NT	2/3	NT	1/5

[1] PDA: potato dextrose agar; [2] OA: oatmeal agar; [3] NT: not tested.

natural cryoprotectants, such as plant substrates or their derivatives, in these ecotopes and by their ability to utilize their inherent mechanisms of protection [10]. Stakhov *et al.* [11] demonstrated that ancient seeds of higher plants provided a specific habitat for microorganisms in frozen ground, thereby favoring their survival for millennia. When bacterial contamination occurs in oomycetes such as *Pythium*, culturing them on OA allows purification of the mycelium that passes through the medium (personal communication from Prof Dr Motoaki Tojo at Osaka Metropolitan University, Japan). This is thought to be because OA is porous and contains a large amount of insoluble fractions, such as plant fiber. Mycelia of LMS could grow during unfrozen winter and reach into substrates, such as dead trees and branches, to tolerate freezing and survive the frozen winter season in northern Japan.

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REFERENCES

1. Akaishi D. [Mushroom fauna of the Ashiu Research Forest: from past literature review and field survey in 2019–2021]. Forest Research, Kyoto. 2023; 82: 1—15. Japanese.
2. Hikuma Y, Miura K, Funatsu S, et al. [Macrofungi collected from Nshikoma experimental forest of Shishu University]. Bulletin Shinshu University Alpine Field Center. 2025; 23: 25—48. Japanese.
3. Kasuya T, Sasaki D, Sasaki Y, et al. [Taxonomic notes on luminescent *Mycena* species recorded from Hitachiota, Ibaraki Prefecture, Japan]. Bulletin of Ibaraki Nature Museum. 2017; 20: 47—50. Japanese.
4. Kitamura M, Takahashi H, Koyama S, et al. [Fungi collected near Toyoni lake]. Erimo Research. 2021; 18: 15—33. Japanese.
5. Kudo S, editorial supervisor, Kawai D. [Oirase river valley mushroom handbook: Spring — early Summer]. Towada: The Society for Oirase Nature and Ecotourism Resources; 2022. 144 p. Japanese.
6. Miyazaki K, Tomikawa Y. [Higher fungi collected in Shimane Prefecture (IX) — New identified native species from 2021 to 2024]. Bulletin of the Shimane Prefecture Mountainous Region Research Center. 2025; 21: 35–42. Japanese.
7. Murakami R, Yajima Y, Kida K, et al, Surviving freezing in plant tissues by oomycetous snow molds. Cryobiology. 2015; 70: 208—10.
8. Oba Y, Hosaka K. The luminous fungi in Japan. J Fungi. [Internet]. 2023 May 26 [cited 2026 Mar 10]; 9: 615. Available from: https://www.mdpi.com/2309-608X/9/6/615
9. Ohsawa M, Hirai N, Kuroda Y, et al. [Flora of higher fungi in university forest at Yatsugatake and Kawakami, University of Tsukuba]. Bulletin of Tsukuba University Forests. 1986; 2: 69—75. Japanese.
10. Ozerskaya S, Kochkina G, Ivanushkina N, et al. Fungi in permafrost. In: Margesin R, editor. Permafrost Soils, Soil Biology. Vol. 16. Berlin: Springer-Verlag; 2009. p. 85—95.
11. Stakhov VL, Gubin SV, Maksimovich SV, et al. Microbial communities of ancient seeds derived from permanently frozen Pleistocene deposits. Microbiology. 2008; 77: 348—55.

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СТІЙКІСТЬ ДО ХОЛОДУ ТА МОРОЗУ БІОЛЮМІНЕСЦЕНТНИХ ГРИБІВ РОДУ *MYCENA* (AGARICALES, BASIDIOMYCOTA) У ПІВНІЧНІЙ ЯПОНІЇ

***Ключові слова*:** адаптація до холоду, кріофільність, гриби, ріст міцелію, психрофільність, психротолерантність, психротроф, температурна залежність.