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Note

Enhancement of ascospore germination from *Aleuria aurantia* after cold storage

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Ascospores of *Aleuria aurantia* did not germinate soon after collection. The treatment with alkali induced germination, but the rate was less than 0.01%. After storage of the ascospore at 4°C or at −20°C for three mo, germination was enhanced, and its rate was approximately 60%.

Key Words——*Aleuria aurantia*; alkali treatment; ascospore germination; cold storage; Discomycetes.

Aleuria aurantia (Fr.) Fuckel is an ascomycete fungus, known as orange peel mushroom, that belongs to Pyronemataceae in the class of Discomycetes. This mushroom is often observed in autumn on freshly broken grounds or edges of forest roads in Japan, and known to be distributed all over the world. In the course of studies on this fungus, we found a phenomenon that a germination rate of the ascospore from *A. aurantia* was enhanced dramatically after cold storage.

Fruit bodies of *A. aurantia* were collected in October, 1998 near Mt. Akagi, Gunma Pref., Japan. As shown in Figs. 1A–C, a fruit body of *A. aurantia* was thin and fragile, and cup-to saucer-shaped with a diameter of 20–100 mm. Hymenium was smooth and bright red-orange. Eight ascospores of 14–16 × 10 mm were observed in an ascus. Paraphyses were slight clavate with thick-endings at their tips. Ascospores were hyaline and elliptical with distinct coarsely reticulate ornamentation. Two droplets were observed inside of an ascospore.

For induction of ascospore germination in some discomycete fungi, a treatment with a certain chemical reagent or with heat was reported to be effective. In *Ascobolus* species, the treatment with dilute alkali followed by incubation at 37°C induced germination at the rate of 80–90% (Yu, 1954). In *A. aurantia* the treatment with nonyl-alcohol was reported to induce germination, but the rate was only less than 1% (Paden, 1972). In this study ascospores of *A. aurantia* were collected from mature apothecia puffing on a sheet of paper. A piece of this paper was washed in distilled water, and the suspension was stored at 4°C or at −20°C for 3 mo. Aliquots of the suspension were transferred onto potato dextrose agar (PDA)-plates (Nissui) containing 100 μg/ml of chloramphenicol (Sigma). The plates were incubated at 20°C for 4–7 d in the dark. A number of germ tubes appearred was counted microscopically and the rate of germination was calculated. For alkaline treatment, the spore suspension was brought to 0.2 N of KOH and allowed to stand for 20 min, after which aliquots of the suspension were transferred onto PDA-plates.

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Table 1. Germination of ascospores of *A. aurantia*.*

Expt. No.	Treatment	No. of ascospores observed/plate	No. of ascospores germinated/plate	Germination rate (%)
1	no	3.3×10^5	0	0
2	0.2 N KOH for 20 min	3.3×10^5	19±5	<0.01
3	4°C for 3 mo	3.0×10^2	177±57	59±19
4	−20°C for 3 mo	3.0×10^2	183±32	61±11

* Spore suspension with 3.3×10^5 ascospores was spread on each plate. In Expt. 1 and 2, all the ascospores in a plate were observed, and in Expt. 3 and 4, randomly 300 ascospores/plate were observed microscopically. Plates were incubated at 20°C for 7 d (Expt. 1 and 2), and for 4 d (Expt. 3 and 4), respectively. No. of ascospores germinated/plate was the average of 5 plates.

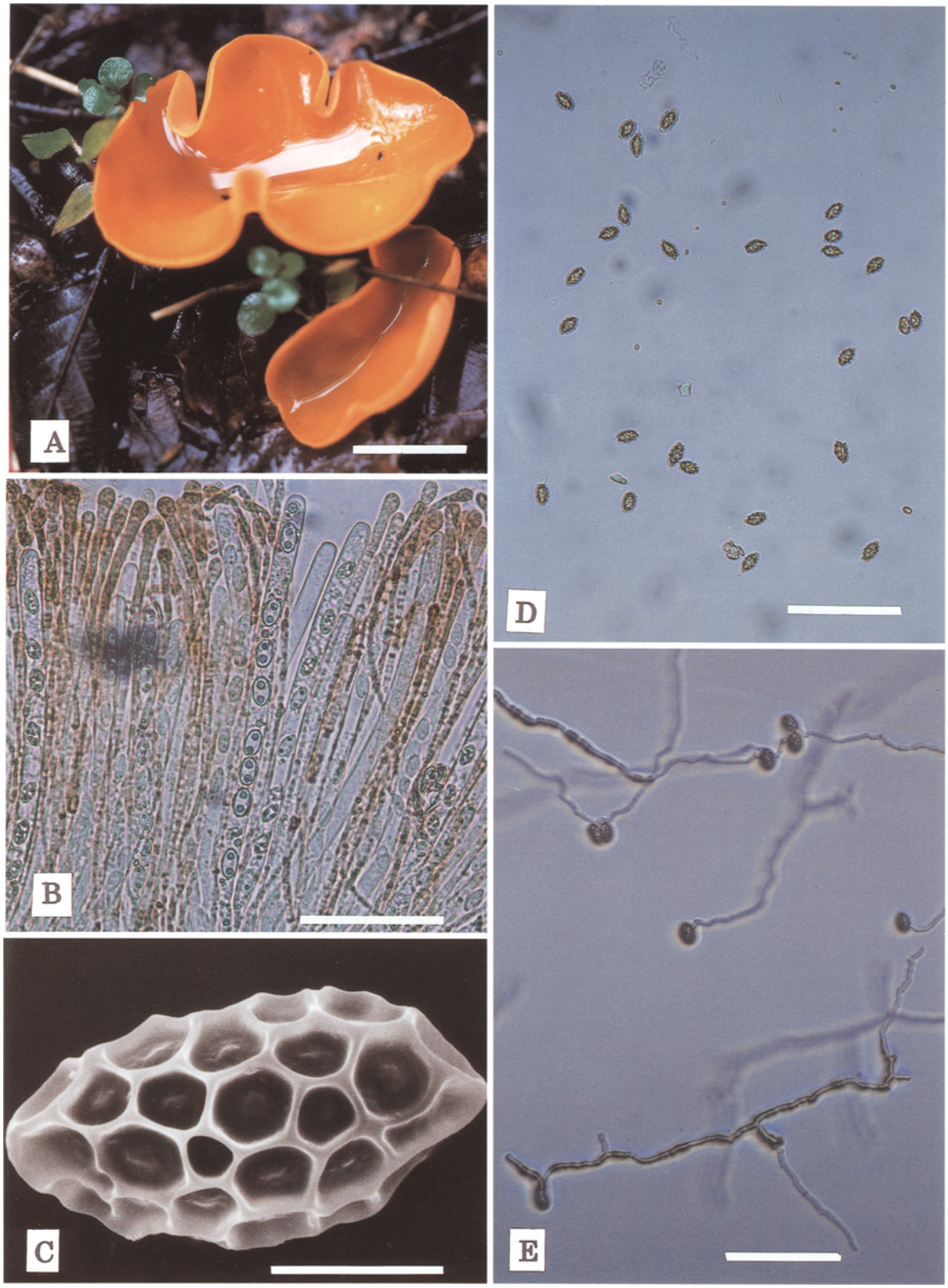

Fig. 1. Fruit bodies, asci and ascospores of *A. aurantia*.
A. Fruit bodies (bar: 20 mm). B. Asci (bar: 80 mm). C. Ascospore (bar: 6 mm). D. Ascospores without any treatment (bar: 80 mm). E. Ascospores after cold storage (bar: 80 mm).

As shown in Fig. 1D and Table 1, the ascospore did not germinate soon after collection. When ascospores were treated with 0.2 N KOH for 20 min, a few germinated after 7 d of incubation but the rate of germination was very low (0.01%). On the other hand, ascospores that had been stored at 4°C or at −20°C for 3 mo, germinated after 4 d at the rate of approximately 60% (Fig. 1E; Table 1). As the same rate of germination was observed after storage at 4°C and at −20°C, this phenomenon did not seem to be due to maturation of ascospores during storage, and the cold storage itself should be effective. The results suggest that the ascospore of *A. aurantia* is necessary to experience cold condition before germination, and the dormancy of the ascospore may be broken by the cold storage. Since the fruit body of this fungus was formed at the end of autumn, and Hwang (1968) noted that mycelia of some discomycete fungus were weak in freezing, our speculation seem to be reasonable for these fungi that survive as ungerminated ascospores during winter.

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