Behaviors of the Saprophytic *Tricholoma matsutake* Mutants G1 and Ar 59 In Vitro Substrate Cultivation: the former exhibited Morphological Changes while the latter did not†

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Tricholoma matsutake produces a specialty mushroom "*matsutake*" only in a symbiotic association with live trees in the wild, unlike cultivated mushrooms based on wood-decaying fungi. Another ectomycorrhizal mushroom *Lyophyllum shimeji* is commercially cultivated to produce the gourmet mushroom "*shimeji*" using spawn substrates comprising barley and saw dust for a few decades.[1)] The key to successful *L. shimeji* fruiting is that the isolates that can grow as spawn without host plants and easily produce fruiting bodies using a protocol similar to that used for cultivated mushrooms.[1)]

We previously reported *T. matsutake* mutants G1 and Ar 59; the former was isolated after irradiating the wild-type NBRC 33136 (a.k.a., Y1) with γ-rays, and the latter was isolated after irradiating the wild-type with an argon-ion beam.[2)] Both mutants exhibited significantly higher amylase and cellulase activities compared with the wild-type; however, G1 gained more saprophytic traits, and it became lethal rather than symbiotic to *Pinus densiflora* seedlings, whereas Ar 59 remained symbiotic. Because of such phenotypic conversions, we examined if these mutants could exhibit some morphological changes relevant to fruiting body production.

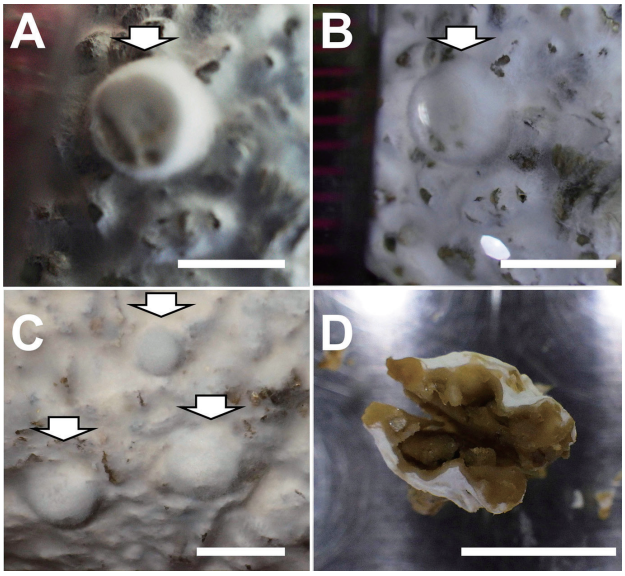

Fig. 1. Morphological changes in *T. matsutake* G1 after substrate cultivation at 23°C for 3 months followed by 16°C for 6 months. (A–C) Lumps arising from the spawn are indicated by arrows. (D) Cross-section of lump indicates tissue differentiation. *Scale bars* 5 mm.

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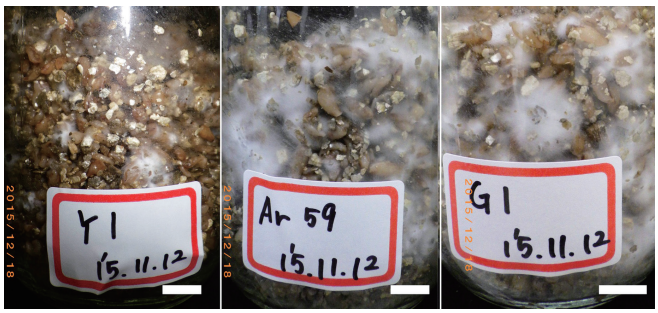

Fig. 2. Mycelial growth of *T. matsutake* NBRC 33136 (= Y1), Ar 59, and G1 after the substrate cultivation for ca. 1 month. *Scale bars* 10 mm.

The *T. matsutake* mutants G1 and Ar 59 and their wild-type NBRC 33136 were separately cultivated with a modified barley-based substrate. G1 developed several tiny (∼5–7 mmϕ) lumps arising from the spawn (Fig. 1A–C). Cross-sections of the lumps indicated that they were not simple aggregates of aerial hyphae but tissue-like, although some uncertainty remains as to whether the lumps are related to fruiting bodies such as a remnant of the inner veil around the pileal margin. The lumps occurred in the G1 spawn in three consecutive independent experiments did not grow into fruiting bodies. Like G1, Ar 59 grew better in the substrate than the wild-type (Fig. 2); however unlike G1, it did not exhibit any morphological changes as observed in NBRC 33136.

The lumps developed in the G1 spawn consecutively in the three independent experiments, and they developed sporadically for nearly 2 years, while other traits of G1 that characterize this mutant, including colony morphology on agar plates, increased degrading enzymatic activities, and harmful effects on plants, were maintained.[2)]

Morphological changes associated with sexual reproduction requires an environment desirable for such vital life cycle events including nutrient availability, temperature, and moisture fluctuations. In the habitat of *T. matsutake*, such moisture and temperature fluctuations occur in the soil and in the atmosphere, even within a single day. To resolve this problem, we are currently inducing mutations in *T. matsutake* G1 by irradiating with heavy-ion beams so that we can obtain mutants that can easily enter the sexual reproduction stage and fruit in artificial cultivation only with substrates. This line of irradiation breeding can eventually yield cultivars of *T. matsutake*.

References

1) A. Ohta, Mycoscience **35**, 147 (1994).
2) H. Murata *et al.*, Botany **97**, 463 (2019).