Argon-ion-beam mutagenesis of the plant-symbiotic edible mushroom *Tricholoma matsutake*†

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Tricholoma matsutake is a filamentous fungus that produces prized mushrooms "matsutake" in association with conifers.[1,2] Currently, no cultivars allow the fungus to fruit artificially, unlike commercially available edible mushrooms. Developing cultivars that are suitable for spawn cultivation will greatly contribute to the artificial cultivation of the plant-symbiotic mushrooms. We hypothesized that irradiation breeding could help produce *T. matsutake* cultivars for fruiting during spawn cultivation. In the present study, as a prerequisite for generating *T. matsutake* mutants, we analyzed the lethality of an argon-ion beam ($^{40}Ar^{17+}$, 95 MeV/u) and generated mutants; this nuclide was used because it has an LET of 280 keV/μm with a theoretical penetrating range in water of 8 mm, which should be sufficient to penetrate the fungal mycelial colony on an agar plate.

An argon-ion beam (0–300 Gy) was irradiated on *T. matsutake* mycelia on agar plates, after which hyphae at the edge of the colony were picked and transferred onto fresh agars, conferring a lethality rate proportional to the radiation dose (Fig. 1). Irradiation with 100–150 Gy accelerated the killing, and that with 300 Gy exterminated the fungi (Fig. 1). *Tricholoma matsutake* strain NBRC 33136, which had fewer aerial hyphae, was more sensitive to the radiation, while NBRC 108262 and NBRC 112911 with more aerial hyphae were somewhat resistant (Fig. 1). No putative mutants were obtained based on mycelial morphology during the lethality experiment. Therefore, we irradiated NBRC 33136 mycelia on agar plates at a dose of 500 Gy and picked pieces from an internal portion of the mycelia, rather than hyphae at the edge of the colony; we hypothesized that with such a high dose, the hyphae in less populated areas would be killed completely, while mutants might occur in densely populated areas.

The protocol conferred some putative mutants with colony morphologies different from that of the wild-type strain. Compared with the wild-type strain (Fig. 2a), most of the putative mutants exhibited abnormal traits at the first screening, including thin mycelial colonies (Fig. 2b), increased numbers of aerial hyphae (Fig. 2c), hyphae that were bundled and grew rather straight (Fig. 2d), and hyphae that were erect (Fig. 2e); however, they generally reverted to the wild-type phenotype during the second screening. Despite such a problem, we isolated one relatively stable mutant, which had much fewer aerial hyphae and grew solely in a planar manner on MMN+V8 agar plates (Fig. 2f). While heavy-ion beams may be useful for isolating mutants of *T. matsutake*, maintaining mutants requires precautions to prevent given traits from reverting after repetitively culturing their mycelia.

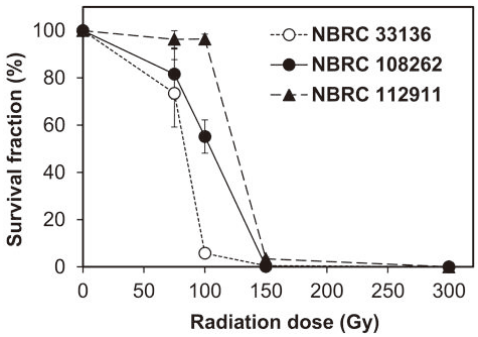

Fig. 1. Lethality of argon-ion beam on *T. matsutake* (n=3).

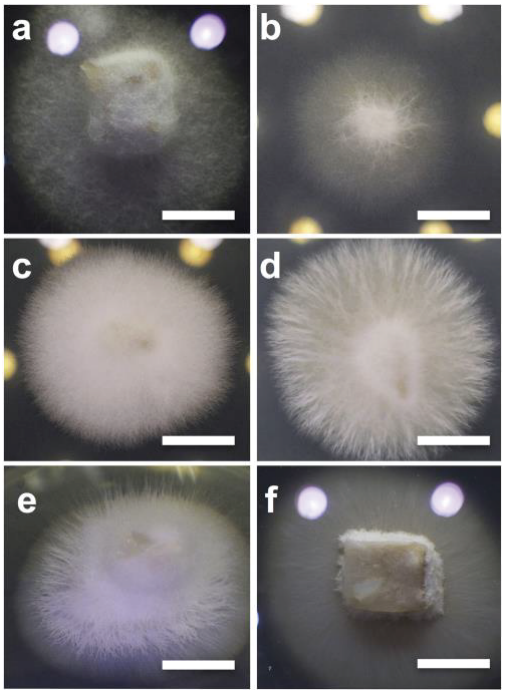

Fig. 2. Mycelial morphology on agar plates of putative *T. matsutake* mutants. **a** The wild-type NBRC 33136. **b–e** Putative mutants whose phenotypes reverted to that of the wild-type after a culture transfer. **f** Putative mutant whose phenotype is relatively stable. Scale bar: 5 mm.

References
1) L. Vaario *et al.*, Mycorrhiza **20**, 511 (2010).
2) A. Yamada *et al.*, Mycoscience **55**, 27 (2014).

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