

## Effect of carbon-ion irradiation on the mycelial growth of *Tricholoma matsutake* in the form of spawn

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*Tricholoma matsutake* is a filamentous fungus that produces gourmet mushrooms, commonly known as “matsutake,” during mycorrhizal association with conifers, most notably red pines *Pinus densiflora* in the Far East Asia and *Pinus sylvestris* in Scandinavia.<sup>1-3)</sup> Currently, neither cultivars nor cultivation methods that allow *T. matsutake* to fruit artificially are available, unlike some wood-rotting edible fungi, such as *Lentinula edodes*, commonly known as “shiitake,” and *Pleurotus ostreatus*, commonly known as “oyster mushroom.” Because we solely harvest the wild fruiting body for trading, the matsutake yield has devastatingly decreased over the past few decades in Japan and other countries that export the commodity. Developing cultivars that are suitable for spawn cultivation to yield matsutake will greatly contribute to countermeasures against overharvesting of the wild mushroom. *Lyophyllum shimeji*, an ectomycorrhizal edible fungus that produces another gourmet mushroom commonly known as “shimeji,” are artificially cultivated for commercial trading after selecting a few extraordinary strains suitable for spawn cultivation.<sup>4)</sup>

Since particle beams have been used widely in crop breeding to generate superb cultivars and industrial microbes with practical uses, we hypothesized that particle beams could be useful in breeding *T. matsutake* cultivars for fruiting during spawn cultivation; note that classical breeding does not work on the fungus because of complex variable nuclear phases and the lack of information on the mating system.<sup>5)</sup> In the present study, as a prerequisite for generating *T. matsutake* mutants, we analyzed the lethality of carbon-ion irradiation and its corresponding doses using the fungal spawn.



Fig. 1. *T. matsutake* spawn. The mycelia axenically cultured in a mayonnaise jar (left) were transferred to a sterile plant tissue culture box (right) for irradiation.

*T. matsutake* mycelia were cultured as spawn in a mayonnaise jar according to a protocol for *L. shimeji* (Fig. 1),<sup>4)</sup> which could be more practical for cultivar selection than agar plate culture. Prior to the irradiation, the spawn was transferred into either a sterile plant tissue culture box (60 × 60 × 60 mm) containing sheets (10 mm thick) of hardwood or a sterile plastic Petri dish (90 × 15 mm) so that the particles pass through the spawn (Fig. 1). After irradiation, the spawn was divided into 10 portions as seeds, inoculated into fresh sterile substrates, and incubated at 22 °C for 60 days (Fig. 2). The extent of mycelial growth in the spawn was scored (Fig. 2).

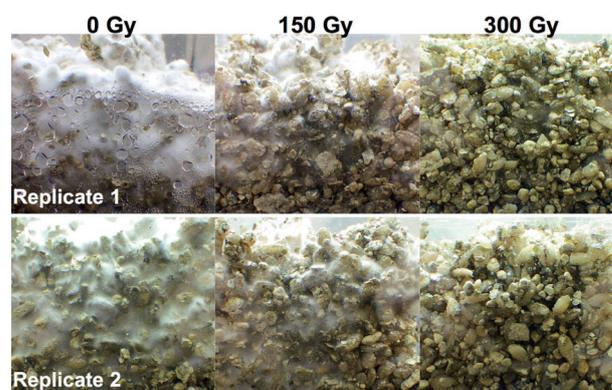


Fig. 2. *T. matsutake* growth after the carbon-beam irradiation. The irradiated spawn was divided as seeds, inoculated into fresh substrates and incubated for 60 days.

The 300-Gy irradiation dose resulted in little growth, as the fungal mycelia only colonized the inoculated area on the surface, while the 150-Gy dose resulted in growth to a certain extent by thinly invading the substrate (Fig. 2). In the controls, the fungal mycelia grew well throughout the substrate (Fig. 2). Therefore, we conclude that the carbon ion irradiation may be useful in generating mutants from *T. matsutake* that is cultured as spawn with a barley grain-based substrate.

### References

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