Particle beam radiation of the ectomycorrhizal basidiomycete *Tricholoma matsutake* that produces the prized, but uncultivable, mushroom "matsutake"

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Tricholoma matsutake is an ectomycorrhizal basidiomycete that produces the economically important edible mushroom "matsutake" in association with Pinaceae plants. The annual, worldwide yield of matsutake is estimated to be 2,000 t, which represents a total retail value of over US$500 million. In Japan, the annual yield of matsutake in the past decade ranged from 50 to 150 t per year, which is much lower than previous annual yields, which reached 12,000 t in 1941. Such a concerning downward trend in the matsutake yield has also occurred elsewhere, including in South Korea, the northeastern provinces of China, and Bhutan.

Mushrooms are fruiting bodies (i.e., the sexual reproductive stage) that contain a tremendous amount of basidiospores. In general, homokaryotic basidiospores are produced after meiosis in dikaryotic hyphae, which result from mating between two monokaryotic hyphae, or between monokaryotic and dikaryotic ones[1,2]. While some ectomycorrhizal mushrooms that are regarded as early colonizers frequently propagate and expand their colonies through spore dispersal, thereby heavily relying on sexual reproduction, and spread elsewhere, those regarded as late colonizers, such as *T. matsutake*, expand their colonies through vegetative hyphal growth, thereby persisting in a preexisting area. However, it has been proven recently that late colonizers also require spore dispersal, thus making them genetically mosaic[3].

Currently, no method is available to cultivate *T. matsutake* to produce fruiting bodies, although methods for artificial mycorrhization and shiro formation have been established in vitro. In fact, in axenic in vitro culture systems, *T. matsutake* associates with a broad range of tree species, including those that naturally harbor arbuscular mycorrhizal fungi, albeit via a root endophytic association, as well as with *Betula platyphylla*, a deciduous broad-leaved tree that shares the same habitat as *Pinus densiflora* and *Tsuga sieboldii*[4]. The most important issue in matsutake cultivation may be the lack of any suitable cultivars. There is a precedent for developing cultivars of fungi that are difficult to cultivate, as the ectomycorrhizal mushroom *Lyophyllum shimeji* is now commercially cultivated to produce the prizedstrains from nature that grow well in a barley-based spawn. Such *L. shimeji* strains were further bred to generate cultivars that were suitable for large-scale mushroom production[5]. Therefore, the creation of strains that are suitable for fruiting, or even spawning like *L. shimeji*, is a key factor for the artificial cultivation of matsutake.

In the present study, we determined whether heavy particle beams could be used to isolate *T. matsutake* mutants. Because we previously obtained some mutants whose traits may be desirable for spawn cultivation and which exhibited a survival rate of ca. 30% following gamma-ray irradiation (500 Gy), we used Ar- and Fe-ions at radiation doses ranging from 0 to 500 Gy. Of these heavy ion beams, Ar irradiation (400–500 Gy) resulted in a survival rate of 12%, while Fe irradiation (large particles, 100–300 Gy) resulted in a survival rate of ca. 65%; following irradiation, some mutants exhibited abnormal mycelial morphologies on an agar plate at the first screening (Fig. 1). However, such mutant phenotypes reverted to the wild-type during the second screening. This phenomenon may be attributed to the fact that we picked a piece of mycelia, rather than a single hypha, wherein the survivor with the wild-type phenotype was mixed with, and eventually overtook, the mutant mycelia. Another issue is that the *T. matsutake* hypha is composed of multinucleated cells, and even a monokaryon isolated from a single spore becomes multinucleated during vegetative growth[6]. Therefore, we conclude that irradiation with heavy particle beams is useful for developing cultivars for matsutake fruiting, although some issues regarding how to obtain stable mutants need to be addressed.

Fig. 1. A putative mutant generated by Fe-ion radiation (300 Gy: left) that generates a large amount of aerial hypae unlike the wild-type (right)

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