Pleiotropic mutant of plant-symbiotic edible mushroom *Tricholoma matsutake* induced by argon-ion beam†

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Tricholoma matsutake is an ectomycorrhizal fungus that produces prized mushrooms "matsutake" in association with conifers.[1] Currently, there are no fungal strains as useful in cultivating fruits as commercial saprophytic edible mushrooms. Developing the strains that are suitable for spawn cultivation will greatly contribute to the artificial cultivation of mycorrhizal mushrooms. Previously, we reported that an argon-ion beam ($^{40}Ar^{17+}$, 95 MeV/nucleon) efficiently killed *T. matsutake* strains on agar plates at a dose of over 100 Gy and generated some mutants whose mycelial morphology was different from that of the wild-type.[2] In this study, we document that the irradiation of argon-ion beam on *T. matsutake* induces a mutant that has pleiotropically altered phenotypes in mycelial morphology and degrading enzymatic activities.

The mutant Ar 59 was obtained by irradiating the mycelia of *T. matsutake* NBRC 33136 on modified Melin-Norkrans agar containing 1.5% V8 juice with an argon-ion beam at a dose of 500 Gy. Next, the mycelial colony was separated into pieces and transferred onto fresh agars.[2] The Ar 59 strain formed a hedgehog-like colony on the potato dextrose agar (PDA) containing 0.1% azurin-crosslinked (AZCL)-amylose and 0.1% AZCL-hydroxyethyl (HE)-cellulose substrates, unlike the wild-type strain that had a flat mycelial mat with flower-like areal hyphae on the inoculation plug (Figs. 1 (a–d)). The Ar 59 strain exhibited a significantly higher amylose- and cellulose-degrading activities than the wild-type strain. In addition, clear halo zones, resulting from the conversion of water-insoluble dye-linked substrates into water soluble substances, were formed around the colonies during the incubation period of 21 days. These clear halo zones extended for seven additional days (Figs. 1 (e–h)).

The phenotype of strain Ar 59 differed substantially from those of *T. matsutake* NBRC 33136, which raises a concern whether Ar 59 is a contaminant. Therefore, the relationship between the NBRC33136 and Ar 59 strains was clarified by analyzing the sequences of their rRNA gene ITS (867 bp) and IGS1 (361 bp) regions, and the phylum-specific mobile DNA *megB1* (479 bp). The results showed that the strain sequences were identical. Therefore, strain Ar 59 was proven to be a mutant of *T. matsutake* NBRC33136.

We have isolated a pleiotropic mutant whose colony morphology differs from that of the wild-type *T. matsutake* strain NBRC33136 and has acquired high levels of amylose- and cellulose-degrading activities. Its pleiotropic feature indicates that argon-ion beams may have deleted a part of negative regulatory region that controls various traits. Whether heavy-ion beams that break double-stranded regions of DNA ranging in size from a few bp to over 1 kilobase positively influence the life cycle of fungus, rendering fruiting in spawn cultivation, remains to be clarified. However, irradiation with heavy-ion beams may be useful to create new traits in *T. matsutake*.

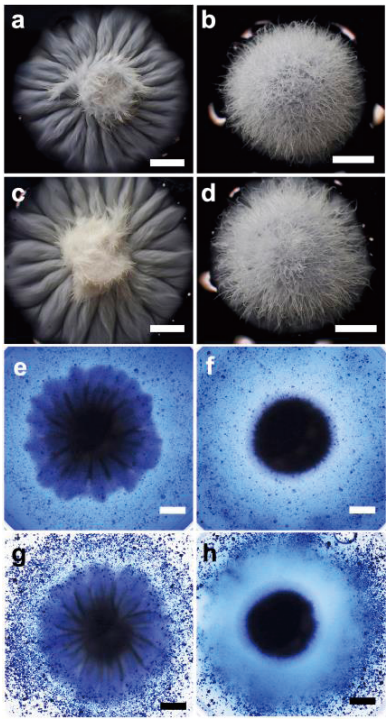

Fig. 1. Traits of *T. matsutake* mutant Ar 59 on PDA containing AZCL-amylose and AZCL-HE-cellulose. (a–d) Colony morphology. (a, c) NBRC 33136 on PDA containing AZCL-amylose and AZCL-HE-cellulose, respectively. (b, d) Strain Ar 59 on PDA containing AZCL-amylose and AZCL-HE-cellulose, respectively. (e–h) Depolymerizing activities. (e, g) NBRC 33136 on PDA containing AZCL-amylose and AZCL-HE-cellulose. (f, h) Strain Ar 59 on PDA containing AZCL-amylose and AZCL-HE-cellulose, respectively. Clear halo zones around the colonies were scored as enzymatic activities (e–h).

References

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