$^{40}Ar^{17+}$ beam-induced mutants of the mycorrhizal mushroom *Tricholoma matsutake* defective in β-1,4 endoglucanase activity better promote the *Pinus densiflora* seedling growth in vitro than the wild-type strain†

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Unlike wood-decaying fungi, ectomycorrhizal fungi have limited plant biomass degrading enzymes, which are mostly hydrolytic enzymes.[1)] The model ectomycorrhizal fungus *Laccaria bicolor* requires its sole cellulolytic enzyme β-1,4 endoglucanase as an effector for cell-wall remodeling during the ectomycorrhizal association with *Populus tremula* × *P. alba*.[1)] Unlike *L. bicolor, Tricholoma matsutake*, which is another ectomycorrhizal fungus that produces "*matsutake*" in association with live pine trees, has many degrading enzymes such as β-1,4 endoglucanase, β-glucosidase, α-gluculonidase, xylanse and β-xylosidase.[2)] In the present study, we examined how *T. matsutake* mutants defective in β-1,4 endoglucanase activity behave in association with the natural symbiotic partner *Pinus densiflora* in vitro.

T. matsutake mutants Ar 5002 and Ar 5012 were generated by the argon-ion beam ($^{40}Ar^{17+}$, 160 MeV/nucleon) irradiation of *T. matsutake* NBRC 33136. Both mutants were defective in β-1,4 endoglucanase activity as per an agar plate assay that uses potato dextrose agar containing 0.1% azurin-crosslinked (AZCL)-hydroxyethyl (HE)-cellulose (Fig. 1). *T. matsutake* has 11 potential cellulase genes, at least one of which is predicted to encode β-1,4 endoglucanase; the rest are classified in the same carbohydrate-active enzyme database (CAZy) family based on the JGI whole genome information, which indicates a mutation could have occurred in a regulatory region.

The wild-type strain NBRC 33136 significantly promoted *P. densiflora* growth in vitro compared with the no inocula control (Fig. 2). Both *T. matsutake* mutants Ar 5002 and Ar 5012 significantly promoted *P. densiflora* growth compared to the growth achieved with the wild-type. The former confers a significantly higher above-ground plant biomass, as well as total biomass, than that with the wild-type strain, whereas the latter confers a significantly higher above- and below-ground plant biomasses, as well as the total biomass, as shown in Fig. 2. No differences in the numbers of lateral roots or mycorrhizal root tips were noted between the mutant and the wild-type strains. No significant differences in the shoot/root (S/R) biomass ratios were noted between the wild-type and mutant strains, as well. The Hartig nets, a hallmark structure of ectomycorrhizal association, was observed among seedlings associated with the wild-type and mutant strains (Fig. 3).

The data suggest that *T. matsutake* without its own β-1,4 endoglucanase activity exerts improved beneficial effects on *P. densiflora* growth in vitro. However, further clarification is required about whether the symbiotic relationship can be strengthened by such a mutated fungal trait.

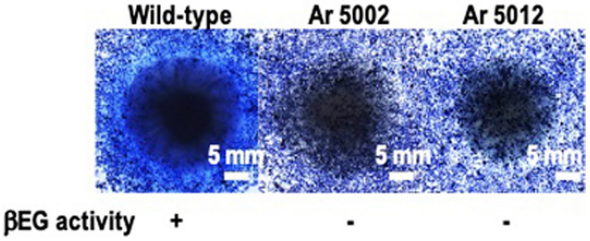

Fig. 1. β-1,4 Endoglucanase (βEG) activities of *T. matsutake*.

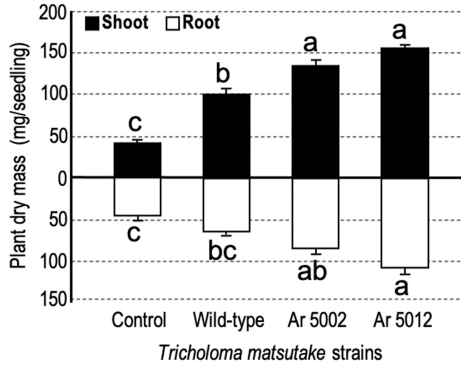

Fig. 2. *P. densiflora* seedling growth after co-culturing with *T. matsutake* strains for 140 d. Bars represent mean ±SEs. The same letters indicate treatments that should be classified as same based on the results of Tukey-Kramer tests.

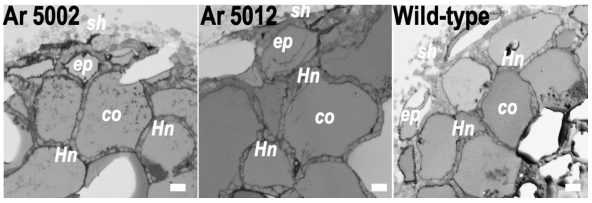

Fig. 3. Cross-section micrographs of the root tissues of *P. densiflora* seedlings associated with *T. matsutake* strains. Abbreviations: co, cortical cell; ep, epidermal cell; Hn, Hartig net; sh, mycelial sheath. *Bars*: 10 μM.

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