Effect of heavy-ion-beam irradiation on mycelial growth of medicinal mushrooms

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It is well known that β-D-glucans from fungi are effective initiators of cell-mediated immunity in humans.[1] β-D-glucans isolated from various fungi differ in their chemical structures and, consequently, in their immunomodulatory activities. For example, schizophyllan, which is a β-D-glucan extracted from the fungus *Schizophyllum commune* and is classified as a medicine, stimulates the immune system.[2] We are aiming to establish beneficial mutants from several extremely rare fungi with immunostimulatory activities. We had already achieved the isolation of several *Cordyceps militaris* mutants with higher proliferative activity by the repeated propagation of expanding hyphal tips from the proton-beam irradiation of the fungus.[3] Heavy-ion beams have been accepted as an effective mutagen.[4] A small number of genes are expected to be disrupted in the mutant genomes because heavy-ion beams can induce mutations with a high frequency at relatively low doses. Such characteristics of heavy-ion irradiation are advantageous for mutation breeding. In the latest study, prior to mutant screening by ion-beam breeding, we examined the dose dependence of mycelial growth of two fungi against the irradiation of heavy-ion beams and compared the mycelial-growth effect of heavy-ion-beam irradiation to that of proton-beam irradiation.

The microbial strain *Fuscoporia obliqua* NBRC 8681 was obtained from the National Institute of Technology and Evaluation. It was inoculated on an agar plate (potato dextrose agar [PDA[5]]) and incubated at 25°C. The microbial strain *Polyporus umbellatus* ATCC 60546 was purchased from the American Type Culture Collection. It was inoculated on an agar plate (yeast malt peptone agar [YM[5]]) and incubated at 25°C. These samples were inoculated on fresh 6 cm plates 4 days before the irradiation and incubated in the dark at room temperature. The 200-MeV proton-beam irradiation (LET 0.5 keV/μm) on the culture plates was carried out by the Wakasa Wan Multi-purpose Accelerator with Synchrotron and Tandem at the Wakasa Wan Energy Research Center in the dose range of 100–2,000 Gy. The culture plates were irradiated with Ar ions (LET 180 keV/μm) and Fe ions (LET 640 keV/μm) in the dose range of 100–300 Gy at the RIKEN RI-beam factory.

For the growth-rate measurement, three loops of mycelia from the center of each irradiated plate were inoculated on each center of three fresh 9 cm plates and incubated at 25°C. The maximum and minimum diameters of the mycelial zone of each plate were measured using a vernier caliper 8 days later. The data from three irradiated plates were averaged and compared with the data from the control.

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Table 1. Effect of ion-beam irradiation on mycelial growth of medicinal mushrooms.

Strain	Ion	Dose (Gy)	Relative ratio of mycelium growth (%)
F. obliqua	H	0	100.0
		500	47.4
		1,000	23.7
		1,500	3.1
		2,000	0.0
	Ar	0	100.0
		150	67.6
		300	50.8
	Fe	0	100.0
		100	82.0
		200	60.7
		300	42.2
P. umbellatus	H	0	100.0
		500	85.7
		1,000	52.7
		1,500	6.2
		2,000	0.0
	Ar	0	100.0
		150	99.1
		300	5.3
	Fe	0	100.0
		100	94.2
		200	56.8
		300	2.3

Table 1 lists the relative ratio of the mycelial growth for two strains, which were irradiated with three kinds of ion beams, respectively. Under any irradiation condition, the mycelial growth rate decreased as the irradiation dose increased. For proton-beam irradiation, the results for irradiation doses of 100 and 300 Gy were almost the same as those of the control (data not shown), and the relative suppressions of mycelial growth were observed from 500 to 2,000 Gy. Relative ratios of mycelial growth of 50.8% and 42.2% were obtained with *F. obliqua* after irradiation with 300 Gy of Ar and Fe ions, respectively. Moreover, relative ratios of 5.3% and 2.3% were obtained with *P. umbellatus* after irradiation with 300 Gy of Ar and Fe ions, respectively. Heavy-ion beams showed a higher relative biological effectiveness (RBE) of mycelial growth inhibition than proton beams, regardless of the kind of fungi.

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