Yield improvement of starch-degrading enzyme in fungi by ion-beam mutagenesis

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To improve the productivity of amylolytic enzymes in filamentous fungi that are widely used worldwide, various breeding processes have been conducted so far. In breeding by classical mutagenesis, chemical mutagen treatment, UV irradiation, and gamma-ray irradiation have been used for years on filamentous fungi; however, the range of mutation could be narrowed with the repeated use of the same conventional methods. Therefore, this study was conducted with the aim of investigating the possibility of heavy-ion beams as a new source of mutagenesis for breeding filamentous fungi.

The spores of *Aspergillus niger* that produce starch-degrading enzymes were dried on membrane filters (ADVANTEC, A020A025A), and these membranes, packed in Hybri-bags (Cosmo Bio), were irradiated with heavy-ion beams of carbon and iron (50 to 400 Gy). The LETs of C and Fe ions were 76 keV/μm and 640 keV/μm, respectively.

The mutants obtained from 200 Gy of C ions and 400 Gy of Fe ions with a survival rate of approximately 20% were examined for their enzyme productivity (Fig. 1).

Simultaneously, we also examined mutants that had been treated with N-methyl-N′-nitro-N-nitrosoguanidine (NTG) and UV irradiation on the same *A. niger* strain and compared them with those obtained using heavy-ion beams.

Among the mutagens used, NTG treatment yielded the most diversified morphology on the strain, and UV irradiation showed the least change in morphology. The morphology of mutants treated with heavy-ion beams was less diversified than that of NTG, but considerable diversity was still observed in the sporulation and the length of aerial hyphae.

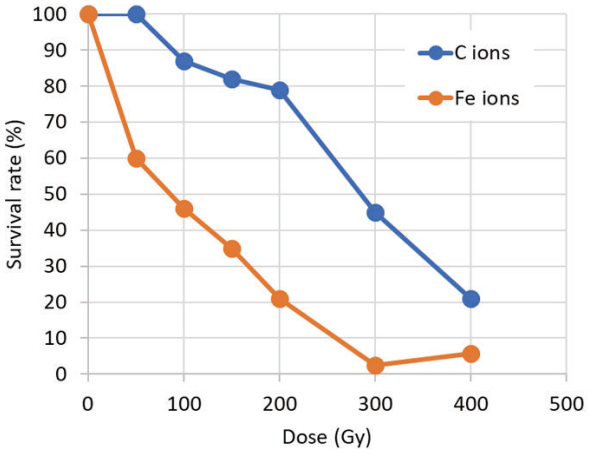

Fig. 1. Survival curves of fungi producing starch-degrading enzymes after C- and Fe-ion irradiation.

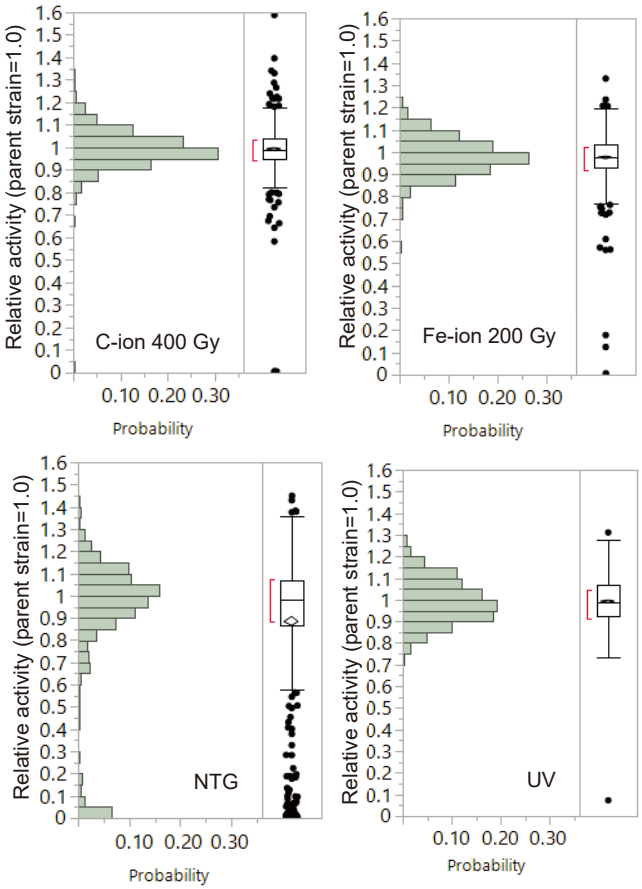

Fig. 2. Yield deviations of mutants treated with various mutagens.

In addition, 920 strains were randomly isolated from each mutagen group and cultivated in a liquid medium with 96-well microtiter plates, and their enzyme productivity was examined and compared with that of the parent strain (Fig. 2). The NTG mutant group showed the largest variation, but both the C-ion and Fe-ion mutants also showed a wide variation. In addition, several mutants from both the ion beams and NTG showed a higher productivity than the parent strain. These mutants will be further examined in larger-scale fermentation.

From these results, it was confirmed that the heavy-ion beam was effective against *A. niger* as a mutagenesis tool for improving productivity. We will continue to conduct screening on a larger scale with the aim of further improving enzyme productivity.

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