

Effect of irradiation ions and doses on the survival rate of yeast

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Yeast has been playing an important role in many human activities, such as winemaking, baking, and brewing for a long period.¹⁾ The budding yeast, *Saccharomyces cerevisiae*, has been considered as the most fundamental eukaryotic model organism. Its small genome size (around 12 Mb), fast growth character, and DNA repair system that is similar to that of plants and animals make it a useful model for gene function and mutation analyses.^{2,3)}

Heavy ion beams cause mutations in the genome, such as deletions, insertions, and base substitutions. Therefore, it has been used as a physical mutagen and to develop new cultivars in the last two decades. Carbon ions have been used frequently because of the high mutation rates and long penetration range, and heavier ions, such as argon and iron, are more suitable for microbial mutagenesis, as they induce larger deletions and insertions, and are more stable than base substitutions.⁴⁾

In the present study, we investigated the dose-survival relationship of a diploid baker's yeast strain Hi1060 (*MATa/α his3Δ1 leu2Δ0 ura3Δ0*). It is genetically equivalent to BY4743 and is established by a cross between BY4741 and BY4742; It is more than three times the size of single-colony isolations on a selective medium. The strain and its parents are all derivatives of the widely used and genome-sequenced laboratory strain S288C. This strain is fully functional for DNA repair by homologous recombination and non-homologous end-joining.

For heavy-ion beam irradiations, Hi1060 was grown in a liquid YPD medium, harvested by centrifugation at $3000 \times g$, and washed three times with 10 mM $MgSO_4$. The cell concentration was adjusted to 1 OD₆₀₀ unit per mL, which is equivalent $\sim 10^7$ cells per mL, with 10 mM $MgSO_4$, and was kept at 4°C before and after irradiation. We irradiated $^{12}C^{6+}$ (135 MeV/nucleon, LET: 23 keV/ μm ; 0–1000 Gy), $^{40}Ar^{18+}$ (160 MeV/nucleon, 189 keV/ μm ; 0–800 Gy), and $^{56}Fe^{24+}$ (90 MeV/nucleon, 647 keV/ μm ; 0–800 Gy). After irradiation, the cells were diluted to different concentrations and spread on YPD plates, and the survival rates were calculated as follows:

Survival rate (%) = num. of the colonies in each dose/non-irradiated control $\times 100$

The determined survival rates and their fitting results by exponential functions are shown in Fig. 1. The results showed that carbon-, argon- and iron-ion irradiations all decreased the survived cell ratio in a dose-dependent manner. The survival curves were well represented by exponential functions with determination coefficients (R^2) of 0.9793, 0.7691, and 0.9509 in carbon, argon, and iron ions, respectively. Ar showed unexpectedly higher and lower survival rates compared with the

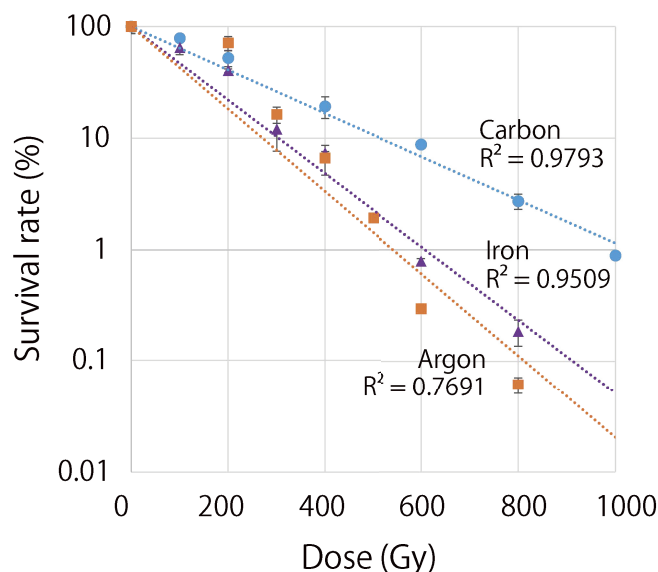


Fig. 1. Dose-survival responses of *S. cerevisiae* Hi1060 (equivalent to BY4743) cells after irradiation by carbon, argon, and iron ions with different doses. The error bars represent standard deviation in technical replicates.

other two ions. We are interested in further investigating Ar irradiations soon.

The lethal effects were more obvious in heavier argon and iron ions than the lighter carbon ions. This is consistent with the previous observations that the relative biological effect of a radiation is higher in heavier ions than the lighter ones. This may reflect that heavier ions, which have higher LETs, induce more deleterious types of mutations to the genome. The doses for 10% survival (D_{10}) were 600 Gy for carbon- and 400 Gy for argon- and iron-ions, respectively. These D_{10} values were in a comparable range to a previous yeast breeding project, where 400 Gy irradiations with carbon and iron ions were used to select high aromatic compound-producing sake yeasts.⁵⁾ Currently, we have been preparing to perform whole-genome sequencing of these irradiated yeast cells. This will quantitatively describe the mutagenic effect of different ions on yeast genomes. At the same time, it would help to understand the relationship between irradiation conditions, survival rate, and the resulting genomic mutations in a base-pair resolution in a genome-wide manner.

References

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