Effects of Ar-ion beam irradiation on survival rate of *Aurantiochytrium* sp.

K. Ikeda,*1 R. Kose,*1 T. Okuda,*1 Y. Sakakura,*1 K. Kaya,*1 Y. Kazama,*2 and T. Abe*2

The organisms that belong to the *Aurantiochytrium* sp. are heterotrophic marine thraustochytrids with a high growth rate. They produce many bioactive compounds such as squalene, docosahexanoic acid (DHA, $C_{22:6n-3}$) and odd-numbered fatty acids, and they are being improved in terms of their cellular cell functions for industrial utilization[1,2].

To introduce novel cell functions, irradiation with a swift, heavy-ion beam to induce mutations is an effective method[3]. At RIKEN RI Beam Factory (RIBF), microbial cells and plant seeds are irradiated with heavy-ion beams such as of carbon and argon (Ar).

In this study, *Aurantiochytrium* spp.NB6-3 and SYLR6#3 were selected because of their short lag times and industrial usage. The strains were exposed to accelerated Ar ions with a dose range of 20 Gy to 80 Gy. After irradiation, cells were cultured on GTY medium (2% glucose, 1% Tryptone, 0.5% yeast extract and 1.8% Red Sea Salt)-containing agar plates (ϕ90 mm) at 25°C for 48 h. The colonies were counted using the colony forming unit (CFU) to estimate their survival rates and were isolated for mutant screening. Values were expressed as averages of duplicate CFU experiments. Survival rate was obtained using the formula:

Survival rate (%) = (number of colonies) / (cell number before irradiation) x 100

The survival rates of both strains rapidly decreased with a dose of 20 Gy (Fig.1). The colony-forming cells were isolated from the agar plates and suspended in the sterilized GTY medium. The cell suspension was transferred to the cell-culture plates with 96 wells for primary screening. Cell density was measured using the iMark Microplate Reader (BIO RAD) at 650 nm. During the primary screening, based on the colony color and a higher growth rate, mutant cells were selected and transferred to test tubes for secondary screening. The colonies of *Aurantiochytrium* with high lipid contents showed cells also show orange to brown color. Therefore, to select mutants with high lipid contents, dark orange to brown colonies were picked out from the various mutated colonies.

During the secondary screening, the selected cells were cultured in test tubes with reciprocal shaking at 25°C for 48 h. The cell growth rate was measured using packed cell volume (PCV, %) and was converted to cell number.

Growth rate was calculated using the following equation:

$$\text{Growth rate (hour}^{-1}\text{)} = \ln(X_1 / X_0) / (t_1 - t_0)$$

where X_1 and X_0 are cell densities (cells/mL) at times t_1 and t_0, respectively. During the primary screening, 22 and 12 mutants were selected from the SYLR6#3 and NB6-3 strains, respectively. However, the characteristic features of the selected mutants in terms of their colors and high growth rates completely disappeared.

The effects of other heavy-ion beams on cells are being investigated.

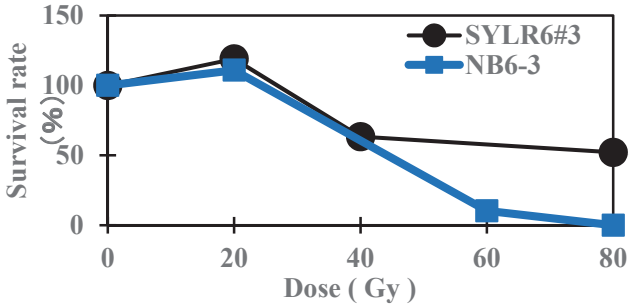

Fig.1 Survival curves of *Aurantiochytrium* sp.

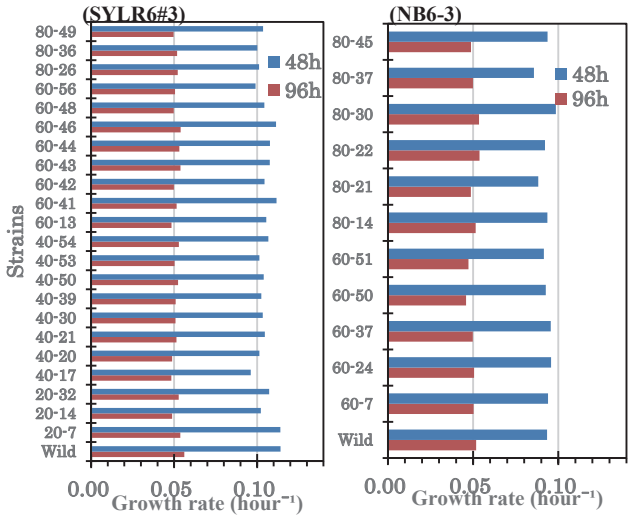

Fig.2 Growth rates of isolated strains estimated during the secondary screening

*1 Sea Act Co. Ltd.
*2 RIKEN Nishina Center

References

1) A. N. Jakobsen et al. : Appl Microbiol Biotechnol. 80 297-306 (2008).
2) Q. Li et al. : Agric. Food Chem., 57, 4267-4272 (2009)
3) A. Tanaka et al. : J. Radiat. Res., 51, 223-233 (2010)