A Novel Radiation-Resistant Yeast, *Filobasidium elegans* RRY1

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1. Introduction

The tolerance to ionizing radiation stress is present among different classes and species of organisms. As listed by Rainey *et al.*, ionizing radiation resistant organisms were isolated from a variety of different sources like processed/canned food items, paper industry, soil and water samples [1]. Apart from extensively reported bacteria and Archea group, many fungal species like *Aspergillus, Curvularia, Alternaria, Cryptococcus, and Ustilago maydis* have been found to be resistant to ionizing radiation [2, 3]. However, different environmental sources are constantly been explored for novel radioresistant organisms, which can help in understanding the molecular mechanism behind these extreme stress responses. On the basis of this, present study was initiated to find novel radiation resistant yeast from sea water source.

2. Methods and Results

2.1 Isolation procedure.

Seawater samples were collected from three places in the West Sea of Korea. Seawater samples (50 ml) were filtered through 4.5 μm pore size cellulose nitrate filter papers and placed face up on isolation medium plates. The plates were incubated at 20°C for 14 days. The colonies developed were purified by streaking and stained with methylene blue to check their shape. Only cultures with a single shape were selected.

2.2 Screening and identification of radiation-resistant yeasts

Selected yeasts were cultivated at 25°C in YPD broth. A 10 μl volume from each cells grown for 7 days were spotted on YPD agar and then subjected to 5 kGy of irradiation at room temperature (RT) using a cobalt-60 γ-ray irradiator. Plates were then incubated at 25 °C for 4 days for colonies formation. Five tolerant strains were found with high radiation resistance. Out of five, the best candidate (RRY1: Radiation Resistant Yeast Strain1) was chosen for further studies. The 18S rRNA gene was sequenced for strain identification. The strain was found to be closely similar to *Filobasidium elegans* (99% similarity).

2.3 Growth and Survival

Growth and survival of *F. elegans* RRY1 was compared with *Saccharomyces cerevisiae* SC7931 and *F. elegans* CBS 7640 strain (procured from CBS-KNAW Fungal Biodiversity centre). All strains were inoculated in YPD broth and growth curve was studied till the stationary phase. The growth profile of RRY1 looked similar to reference strain (Fig. 1A), with an optimum growth temperature of 20°C. For survival studies, log phase cultures were exposed to different doses of gamma radiation and dilutions were plated on YPD agar plates. Plates were incubated at 25°C for 4 days and cfu were counted. *F. elegans* RRY1 strain was found to be far more resistant than *F. elegans* CBS 7640 (Fig. 1B) and *S. cerevisiae* SC7931 (Fig. 1D), with D_{10} value of ~7 kGy (Fig. 1).

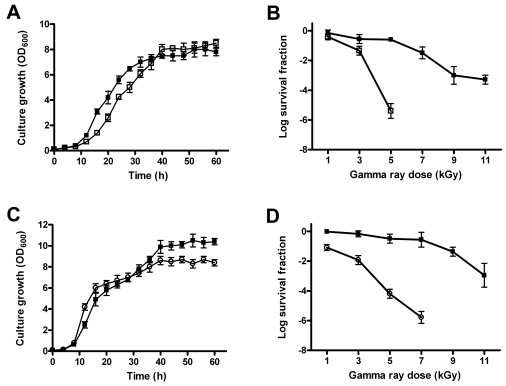

Fig.1. Growth and Survival of *Filobasidium elegans* RRY1, *S. cerevisiae* SC7931 and *F. elegans* ATCC

2.4 Cell Membrane integrity Assay

Log phase cultures of *F. elegans* RRY1 and *S. cerevisiae* SC7931 were subjected 3 kGy of irradiation and processed for PI/Annexin V staining followed by FACS analysis for estimating the viability [4]. As it is evident from Fig. 2, the cell death was very less in *F. elegans* RRY1 strain whereas more than 60% cell death occurred in *S. cerevisiae* SC7931 after 3 kGy stress, indicating its higher resistance to ionizing radiation.

2.5 Cell morphology

S. cerevisiae SC7931 and *F. elegans* RRY1 cells were

subjected to 3 kGy of ionizing radiation and processed for the SEM imaging. *S. cerevisiae*SC7931 cells were found to be distorted whereas RRY1 strain cells were intact and were looking morphologically similar to unirradiated control (Fig. 3).

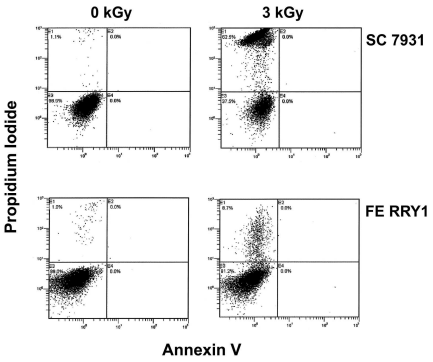

Fig.2. Cell Membrane integrity assay for *S. cerevisiae* SC7931 and *F. elegans* RRY1

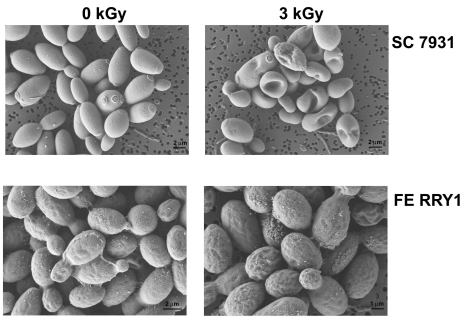

Fig.3. Cell morphology of *S. cerevisiae* SC7931 and *F. elegans* RRY1 under Scanning electron microscope

2.6 DNA damage and repair kinetics

The extent of DNA damage and repair kinetics of *S. cerevisiae* SC7931 and *F. elegans* RY1 strain was performed using Pulse field gel electrophoresis (PFGE). Both *S. cerevisiae* SC7931 and *F. elegans* RRY1 log phase cultures were subjected to 3 kGy of gamma irradiation and allowed to recover in YPD broth under standard growth condition. Samples were collected at different time point of recovery and processed for PFGE [5]. The unirradiated culture samples showed fourteen distinct DNA bands, whereas in irradiated samples, profile was lost because of severe DNA damage occurred under 3 kGy irradiation conditions (Fig. 4). During recovery, the DNA smear started disappearing in *F. elegans* RRY1 strain after 2 hrs of growth and by 3 hrs the lost DNA profile was fully restored back. On the other hand *S. cerevisiae* SC7931 strain was not able to restore back its severely damaged DNA and profile was not restored even after 4 hrs of growth. The superior DNA repair kinetics again proved the radioresistant property of *F. elegans* RRY1 strain.

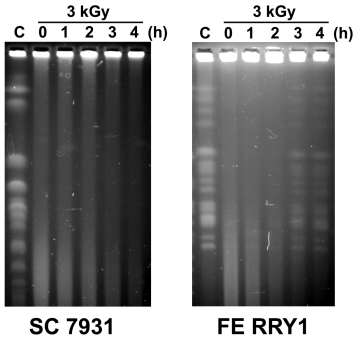

Fig.4. DNA damage and repair kinetics of *S. cerevisiae* SC7931 and *F. elegans* RRY1 using PFGE.

3. Conclusions

A novel radioresistant yeast was isolated from seawater. Based on 18S rDNA analysis, the strain was found to be closely related to *Filobasidium elegans* and thus named *F. elegans* strain RRY1. Radiation stress studies revealed that RRY1 strain was much more radioresistant (D_{10}: 7 kGy) than *S. cerevisiae* SC7931 (D_{10}: 1 kGy) or the reference *F. elegans* CBS strain (D_{10}: ~2 kGy). Cells exposed to 3 kGy of gamma radiation showed negligible cell death. The RRY1 irradiated cells showed superior DNA repair capability during post-irradiation recovery, thus leading to remarkable growth recovery. The growth and survival properties of *F. elegans* RRY1 are different from *F. elegans* CBS strain thus indicating that it is a radioresistant variant of *Filobasidium elegans* As per our knowledge, this is the first ever report of radioresistant *Filobasidium elegans* strain.

REFERENCES

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