Morphological Changes of Yeast Cells due to Oxidative Stress by Mercury and Radiation

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1. Introduction

The yeast *Saccharomyces cerevisiae* is one of the most important microorganisms employed in industry. Growth rate, mutation, and environmental conditions affect yeast size and shape distributions but, in general, the influence of spatial variations in large-scale bioreactors is not considered. Ionizing radiation induces DNA double strand breaks in the nucleus, In addition, it causes lipid peroxidation, ceramide generation, and protein oxidation in the membrane, cytoplasm, and nucleus [1, 2]. Metal ions are essential to life. However, some metals such as mercury are harmful, even when present at trace amounts. Toxicity of mercury arises mainly from its oxidizing properties. As a metal ion, it induces an oxidative stress or predisposes cells to an oxidative stress, with considerable damage to proteins, lipids and DNA. In this work, we investigated to effect of ionizing radiation (IR) and mercury chloride (Ⅱ) on cell morphology.

2. Methods and Results

2.1 Yeast strain and Medium

A *S. cerevisiae* strain W303-1A *MAT*a {*leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15*} was grown on a rotary shaker in a YPD medium with 1% Yeast Extract (Difco), 2% Bacto Peptone (Difco) with 2% glucose at 30 ℃ for 60 h. For the culture used in the analysis 20 ml of a 24 h grown pre-culture, 6ml of a pre-culture medium was transferred to a 1 liter flask containing a 300 ml fresh medium. Growth was checked spectrophotometrically by measuring the optical density at 600 nm.

2.2 Induction of oxidative stress

S. cerevisiae was treated with ionizing radiation, mercury chloride (Ⅱ), and ionizing radiation combined with mercury chloride (Ⅱ). Non-treated cells were used as a negative control group. Four different dose rates, 100, 400, 800 and 1200 Gy hr^{-1}, were applied to get a total dose of 400 and 800 Gy, respectively. Treatment of mercury was done in the concentration range from 0.1 to 0.9 mM. The half lethal dose of radiation was 400 Gy. In this study, 400 and 800 Gy hr^{-1}were used for the combined treatment of ionizing radiation with mercury chloride (Ⅱ).

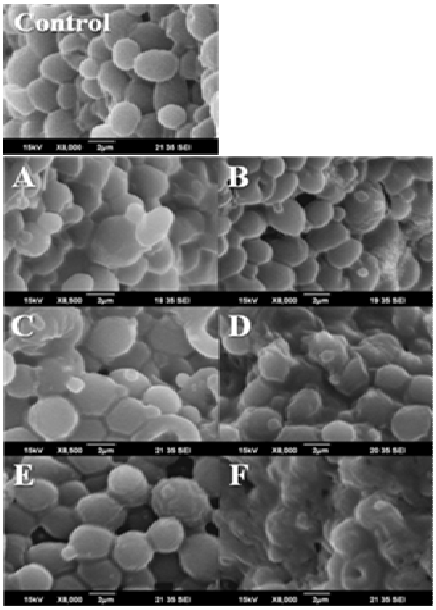

Fig. 1. Morphology of *Saccharomyces cerevisiae* after treatment of mercury chloride (Ⅱ). A. 0.05 mM mercury chloride (Ⅱ) treated cells; B. 0.1 mM mercury chloride (Ⅱ) treated cells; C. 0.15 mM mercury chloride (Ⅱ) treated cells; D. 0.2 mM mercury chloride (Ⅱ) treated cells; E. 0.25 mM mercury chloride (Ⅱ) treated cells; F. 0.3 mM mercury chloride (Ⅱ) treated cells . Non-treated cells were used as a control.

2.3 Image analysis

Image acquisition was conducted using a scanning electron microscope in a vacuum condition. Preprocesses such as washing, fixing, dehydrating, drying and coating were needed before morphological observations [3-5]. The yeast suspension was washed several times. Yeast cells were fixed in a solution of 2 % glutaraldehyde, dehydrated in a graded series of ethanol and acetone concentrations. After the last water droplets had evaporated, but with the cells still visibly moist, the cells were irradiated with the IR laser. Directly following the laser irradiation, a gold layer was sputter-coated onto the sample using a Cressington Sputter coater (Cressington Scientific Instrumets Ltd. England. UK). SEM images were obtained in a JEOL JSM-6390 with digital image capture capability.

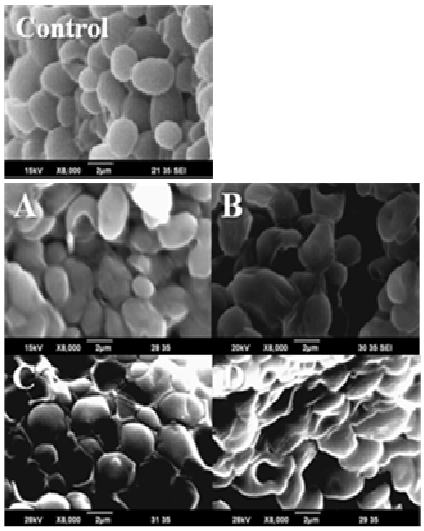

Fig. 2. Morphology of *Saccharomyces cerevisiae* after treatment of ionizing radiation. A. treated with 400 Gy hr^{-1}, 400 Gy ionizing radiation; B. treated with 400 Gy hr^{-1}, 800 Gy ionizing radiation; C. treated with 800 Gy hr^{-1}, 400 Gy ionizing radiation; D. treated with 800 Gy hr^{-1}, 800 Gy ionizing radiation. Non-treated cells were used as a control.

Wrinkled or folded cells with an irregular rough surface were observed after mercury chloride treatment (Fig. 1.). Cell morphology was more modified under a higher concentration of metalloid stress. Ionizing radiation treatment made cells more folded and wrinkled (Fig. 2.). Morphological changes were increased with higher radiation doses.

3. Conclusions

Ionizing radiation and mercury chloride (Ⅱ) could induce oxidative stress in the yeast cells. Higher dose of radiation as well as higher concentration of mercury chloride induced more changes in the cell morphology. However, cell morphology was modified differently according to the type of oxidative stress.

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