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Growth effect of *Saccharomyces cerevisiae* with neutral oxygen-radical treatments
中性酸素ラジカル照射による出芽酵母の増殖効果

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We have focused on the cell cycle in order to investigate the strict mechanism of the effect of oxygen radicals on the promotion of the growth of budding yeast cells. Yeast cells were synchronized at each phase with respective reagents, and treated neutral oxygen radicals. As a result, growth of cells synchronized at G_1 phase was promoted to be 1.1 times with oxygen radical treatment for 10 s. The results suggested that oxygen radicals stimulated the yeast cells at G_1 phase, accelerated the cell cycle and promoted the cell growth.

1. Introduction

Recently, the uses of nonequilibrium atmospheric-pressure plasmas are expected for the biological applications in medical and agricultural fields such as sterilization, blood coagulation, cure of cancers, and growth promotion of crops. We developed an atmospheric-pressure oxygen-radical source, which only produce neutral species.[1][2] We reported the quantitative inactivation effects based on the measurements of the densities of neutral oxygen radicals such as ground-state atomic oxygen [$O(^3P_j)$] and singlet oxygen molecule [$O_2(^1\Delta_g)$], and showed that the $O(^3P_j)$ was the dominant species responsible for the inactivation of *P. digitatum* spores.[3][4] Moreover, we have studied the growth effect of budding yeast cells (*Saccharomyces cerevisiae* W303a) using an atmospheric-pressure oxygen-radical source as well as the inactivation effect.[5] The results suggested that the effects of oxygen radical treatment on the budding yeast cells changed from promotion to repression of the growth, and finally exhibited the inactivation with increasing the treatment time.

Eukaryote commonly proliferates their cells on the basis of cell cycle, which is the series of events to produce two daughter cells composed of DNA replication and cell division. Figure 1 shows cell cycle of budding yeast cells. In the S phase, the chromosomes including genomic DNA replicate. Then, nuclear is equally divided (karyokinesis) and subsequently cytokinesis occurs in the M phase. Besides, two gaps (G_1 and G_2 phase) exist between S and M phases.

In this study, we have focused on the cell cycle in order to investigate the strict mechanism of the effect of oxygen radicals on the promotion of the growth of budding yeast cells. Yeast cells were synchronized at each phase with respective reagents, and treated with oxygen radicals.

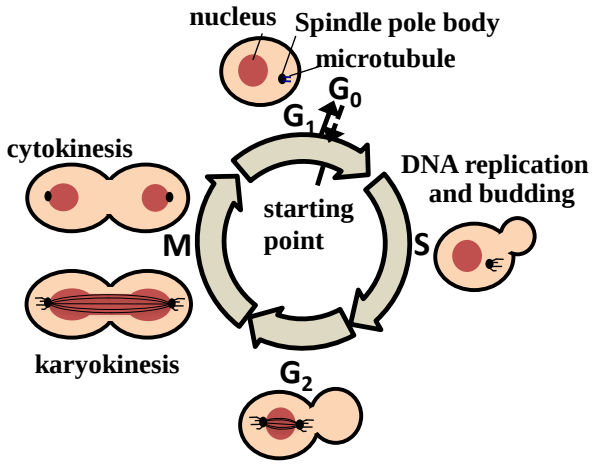

Figure 1 Schematic diagram of cell cycle of budding yeast.

2. Experimental procedure

After the preculture for 18 h in yeast extract peptone dextrose (YPD) medium, yeast cells were diluted 20 times (about 5×10^5 cells/ml), and cultured for 3 h in YPD medium. To synchronize the cells at S, G_1, or M phase, the reagents such as hydroxyurea, α-factor, nocodazole were added, respectively, and the cells were additionally cultured for 3 h. Then, the cells were suspended with phosphate buffered saline (PBS(-)). The suspensions were treated with neutral oxygen radicals. The sample was placed on the movable stage at 4 mm/s, and the radical source head and the

sample were enclosed with a plastic cover, and purged with Ar gas in order to eliminate the influence of ambient air, as shown in Fig. 2. Oxygen radicals were treated under the conditions of an $O_2/(O_2+Ar)$ gas flow ratio of 0.6%, a total flow rate of 5 slm, and exposure distance of 10 mm for 10, 15, and 20 s. After oxygen radical treatment, the treated cells were arranged to be 1.0×10^3 cells/ml, and cultured with YPD medium at 30 °C for 48 h after the treatment. To estimate the growth of yeast cells, the numbers of cells were counted.

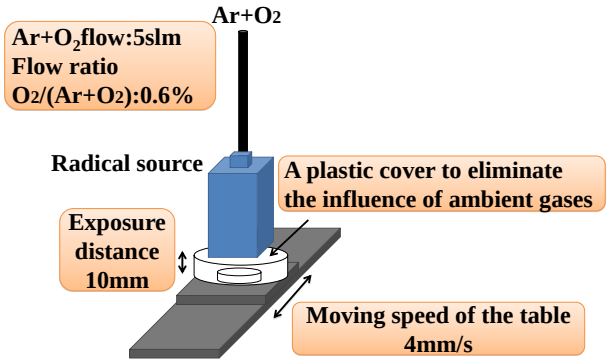

Figure 2 Schematic diagram of oxygen radical treatment.

3. Results and discussion

Figure 3 shows the effects of oxygen radical treatment for 10, 15, and 20 s on the growth of budding yeast cells synchronized at G_1, S, and M phase. The vertical axis shows the ratio of the number of cells to control cells. In the cells synchronized at G_1 phase, the cell growth was promoted to be 1.1 times with oxygen radical treatment for 10 s. And then, the growth was gradually repressed to be about 0.9 and 0.6 times with an increase of the treatment time for 15 and 20 s, respectively. In contrast, the promotion of cell growth with oxygen radical treatment was not shown in the cells synchronized at neither S nor M phase. The ratios of the number of cells to control cells were gradually decreased with an increase of the treatment time such as 10, 15, and 20 s. Therefore, the effect of the oxygen radical treatment on the promotion of the growth of yeast cells was exhibited at only G_1 phase. The results suggested that oxygen radicals stimulated the yeast cells at G_1 phase, accelerated the cell cycle and promoted the cell growth.

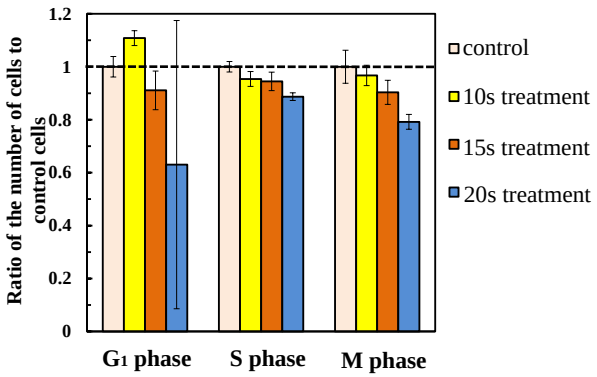

Figure 3 Ratio of the number of cells at various phases after cultured 48 h.

4. Conclusion

To investigate the mechanism of the effects of oxygen radicals on the growth of budding yeast cells, the cells were synchronized at S, G_1, and M phases with the reagents such as Hydroxyurea, α-factor, and Nocodazole, respectively, and treated with oxygen radicals. The results indicated that the promotion of cell growth was shown in the only cells synchronized at G_1 phase. Therefore, the results suggested that oxygen radicals stimulated the molecular mechanism in the cells at G_1 phase and promoted the cell growth.

5. Acknowledgement

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6. References

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