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Evaluation of genotoxicity found in non-thermal atmospheric pressure plasma using yeast cells

大気圧低温プラズマが有する変異原性の酵母細胞による評価

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Non-thermal atmospheric pressure plasmas have been recently applied in biomedical field. So far, the influence of the plasma treatment to living cells and organs is still unknown. In this study, we have monitored DNA damage in yeast cells exposed to argon plasma torch. We have used the yeast-based genotoxicity assay system, which is based on the transcriptional induction of a *Saccharomyces cerevisiae* with a *RNR2-lacZ* reporter plasmid in response to DNA damaging agents and agents that interfere with DNA synthesis. As well as an alkylating agent or UV radiation, argon plasma torch treatment induced the high levels of *RNR2-lacZ* expression. These strongly suggest that non-thermal atmospheric pressure plasmas treatment could cause genotoxic effects via cellular DNA damages.

1. Introduction

To understand the interaction between plasma and living system is essentially important for supporting medical and sanitary use of low temperature plasma [1]. Microorganisms are useful materials to analyze the effects caused by such a plasma exposure [2]. We have been studying the inactivation process of bacteriophages (viruses which infect to bacteria) exposed to atmospheric pressure cold plasma. Phage producing activity of the plasma-treated phage DNA was assayed by transfection into the host *E.coli* cells. It was found that single-stranded phage DNA is extremely sensitive to the plasma comparing to double-stranded phage DNA. It is suggested that plasma-damaged double-stranded DNA is preferentially restored by the cellular DNA repair system which scarcely works for the damage of single-stranded DNA [3].

Another effective approach to the plasma and living system interaction is to study the influence of atmospheric pressure plasma to the gene expression in living organism. Recently, improved genotoxicity tests have been established by harnessing cellular responces to DNA damage. We have constructed a yeast-based genotoxicity test system using reporter assay linked to DNA damage-inducible promoter [4]. The yeast *Saccharomyces cerevisiae* is unicellular, easily manipulated and thus respond to various DNA-damaging agents in a mammalian cells than bacteria do. Application of argon plasma jet to the yeast-based system induced the high levels of the reporter gene expression. Evidence is provided that this reporter system can monitor genotoxic factors included in the atmospheric pressure cold plasma.

2. Experimental part

The schematic of the experimental set up for plasma exposure is shown in Fig.1. The plasma jet generator was constructed with a glass tube, a stainless steel wire, and a stainless steel mesh. The stainless steel wire (0.2 mm in diameter) was fixed as a high voltage electrode coaxially inside the glass tube. The stainless steel mesh (10 mm width) was wrapped at the 5mm from the tip of the glass tube as ground electrode.

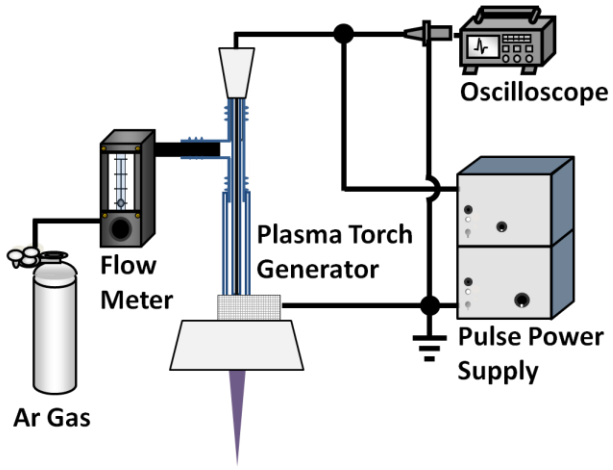

Fig.1 Schematic of the experimental set-up.

Dielectric barrier discharge (DBD) was generated between the both electrodes, using a pulse power supply (ECG-KOKUSAI, PPS-8000). The applied voltage was 12 $kV_{0\text{-}P}$, the frequency was 2.5 kHz, and the pulse width was 2.8 s. The flow rate of argon was 2.0 L/min. The gap between the tip of the generator and the surface of the sample solution was 30 mm.

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3. Results and discussion

Figure 2 shows the concept of the yeast genotoxicity test system. DNA damage on the chromosomes works as a signal to the cell itself which order to induce the high level of *RNR2* gene expression. *RNR2* (ribonucleotide reductase subunit gene) is involved in cellular DNA repair reactions. Therefore, the reporter *lacZ* gene under *RNR2* promoter is induced by DNA damage. The extent of DNA damage was assayed by the reporter β-glactosidase activity of the yeast cells.

Fig.3 shows the results of plasma application to the yeast cells. High levels of *RNR2-lacZ* expression occurred by the argon plasma jet exposure. The results indicate that atmospheric pressure cold plasma has strong genotoxicity. The yeast test system responds to many type of carcinogenic reagents. So, carcinogenic effect of the atmospheric pressure cold plasma is also strongly suggested.

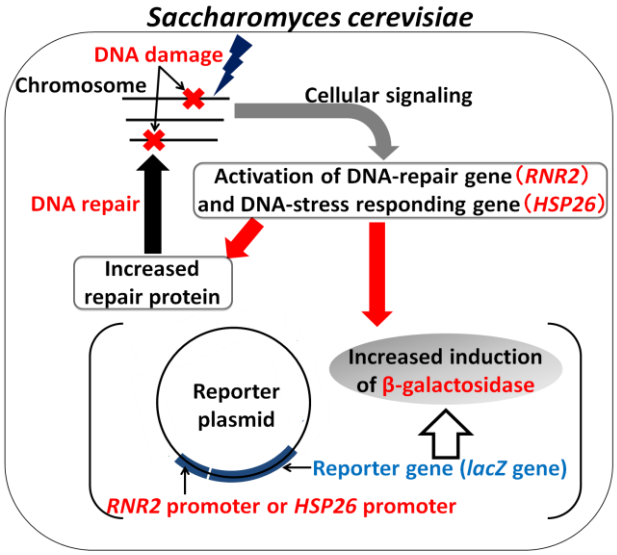

Fig.2 Reporter assay system for detection of DNA damage.

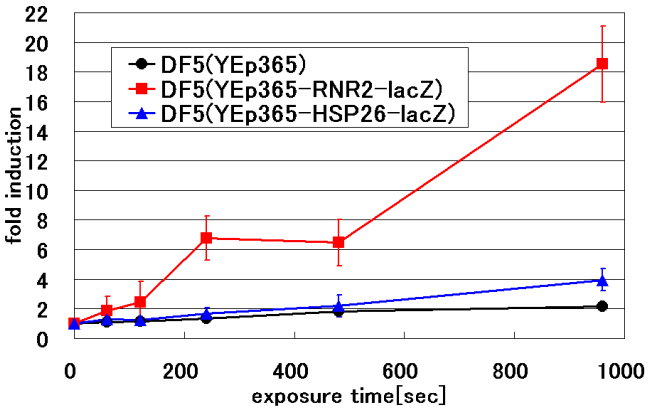

Fig.3 Induction of β-gal activity by argon plasma torch. Yeast strain used DF5 cells with YEp365-*RNR2-lacZ* (filled square), YEp365-*HSP26-lacZ* (filled triangles) and YEp365 (filled circle).

References

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