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Sterilization Effects of Reactive Species in Atmospheric Air Plasma on Plant Pathogenic Fungi

植物病害に対する大気圧空気プラズマ中の活性種の殺菌効果

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The plasma irradiation suppressed the germination of the spores of *Gromerrela cingulata*. To discuss the role of hydroxyl radical (OH*) on the suppression of the germination of *Gromerella cingulata*, the plasma irradiation length and time were varied, and total dose of OH* was measured. It was found that no germination was observed with more than 0.02 nmon/mm^2 of the total dose of OH* at any conditions.

1. Introduction

In the last decade, atmospheric pressure plasma (APP) has been widely used in various ways for medical or agricultural applications. Many groups developed sterilization methods using APP and reported that reactive oxygen species (ROS) and reactive nitrogen species (RNS) irradiated from APP have important physical and chemical effects on the biological tissues, such as gene transfection [1], growth promotion [2], and selective killing of cells [3]. However, there are unexplained matters; such as identification of reactive species dominating the sterilization effect for each plant pathogenic fungus. In this work, we focus on the effect of germination suppression by hydroxyl radical (OH*), one of the ROS, on *Gromerella cingulata*.

2. Experimental setup

2.1 *Atmospheric air plasma*

A plasma jet using air and water has been developed as shown in Fig.1. Influences of two parameters; irradiation length L and total irradiation time T on germination suppression were investigated. To understand the role of the OH* on the sterilization, the concentration of OH* in the plasma irradiation region was measured using terephthalic acid (TA) [4,5].

2.2 *Effects on germination rate of Gromerella cingulata*

Mycelium of *Gromerella cingulata* was pre-incubated on Potato Dextrose Agar (PDA) plate at 28 °C for 5 days. Spores of *Gromerella cingulata* were suspended in distilled water (5.0x10^5 spores/ml), and the suspension of 20 µl on glass plate was irradiated with plasma. After the medium solution was added, the tested spores were grown in the hemocytometer at 28 °C for 6 hours in the dark. The rates of germination of the spores were determined with a phase-contrast microscope and assessed as a percentage of the respective germination in the total number of the spores.

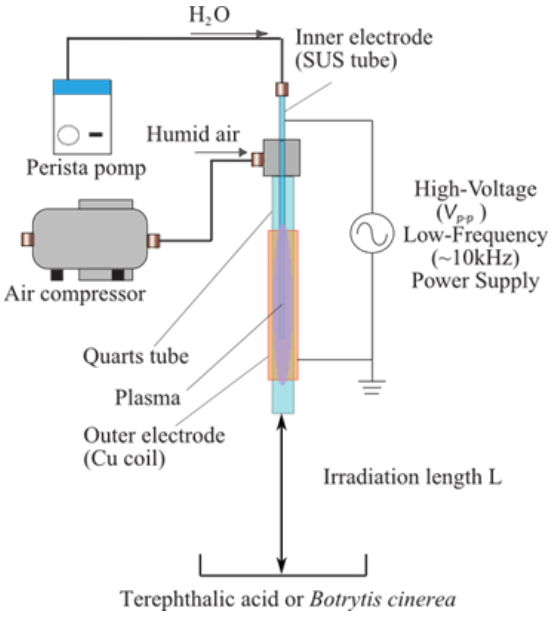

Fig.1. Experimental setup.

3. Experimental Results and Discussion

The spores were irradiated with the plasma varying L and T. Figure 2 shows photographs of *Gromerella cingulata* in the hemocytometer after the cultivation. The left is control (irradiated with air for 60 seconds), and the right is irradiated with plasma for 60 seconds. The spores without the plasma irradiation germinated and mycelium began to grow, while the germination of the spores with the plasma irradiation was suppressed.

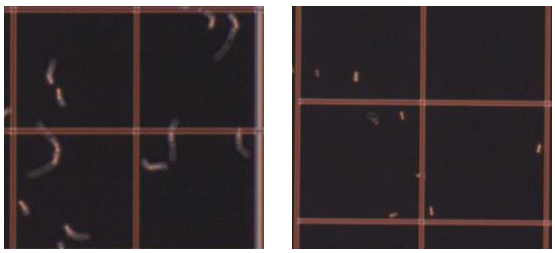

Fig. 2. Germination of *Gromerella cingulata*.
Left : Irradiated with air for 60 seconds.
Right : Irradiated with APP for 60 seconds.

The suppression rate of the germination depended on the plasma irradiation time and length (Fig. 3). At any irradiation length, the germination rate was lower at larger T.

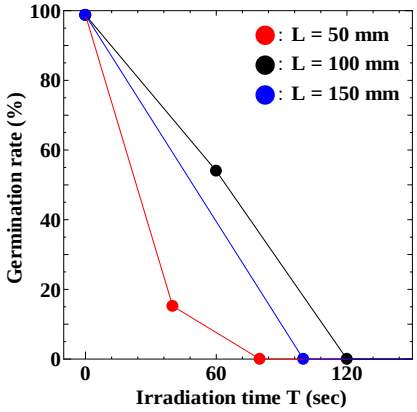

Fig. 3. Effect of irradiation time on germination rate.

Total dose of OH* estimated from the concentration of 2-hydroxyl TA (HTA) is higher at longer T, and lower at larger L (Fig. 4). Due to the constant OH* flux, the total dose of OH* linearly increased against the irradiation time T. Both the short lifetime of OH* and the jet stream expansion resulted in decrease of OH* against the irradiation length L.

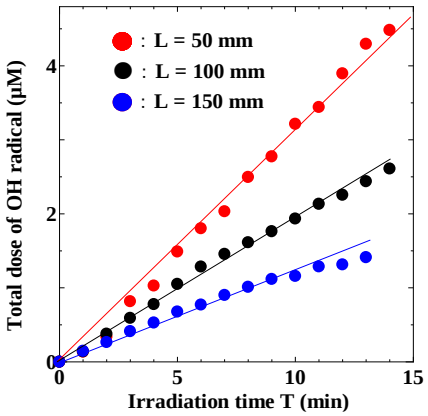

Fig. 4. Total dose of OH radical under various plasma irradiation time and length.

The germination rate is plotted against the total dose of OH* in Fig. 5. It was found that no germination was not observed with more than 0.02 nmon/mm^2 of the total dose of OH* at different length and time. To discuss the role of OH* on the germination suppression of *Gromerella cingulata*, further experiments are undergoing.

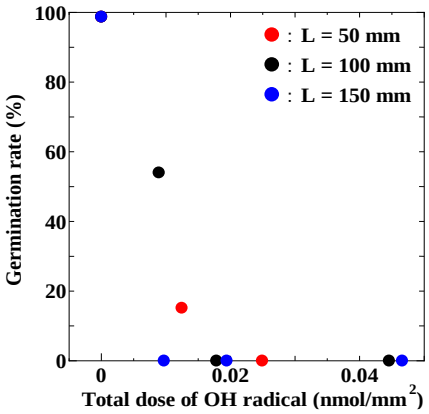

Fig. 5. Effect of total dose of OH radical on germination rate.

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