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Plasma-spore of *P. digitatum* Interactions in Low Temperature Atmospheric Pressure Plasma

低温大気圧プラズマとミドリカビ胞子の相互作用

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The spores were inactivated using an oxygen-radical source that supplies only neutral oxygen radicals. The inactivation rate of *Penicillium digitatum* spores was correlated with the ground-state atomic oxygen [O (^3P_j)]. The result indicates that O (^3P_j) is the dominant species in the inactivation. The effect of plasma treatment on cell membrane of the spores of *P. digitatum* was observed by using fluorescent microscopy with 1,1'-Dioctadecyl-3,3,3',3'-tetramethylindocarboeyanine perchlorate (DiI). The analysis revealed that plasma treatment caused the staining of intracellular organelles, in addition to cell membrane.

1. Introduction

Methods of inactivating microorganisms using a non-equilibrium plasma processing have recently attracted much attention. Non-equilibrium plasma processing has many advantages with respect to low-temperature processing and short processing times. Plasma inactivation has proved to be an effective system that causes minimal damages to the instruments.

Many factors which can possibly cause inactivation, for example vacuum ultlaviolet (VUV) and UV-C emissions, neutral and charged species, electric fields, and their synergistic effects, have been studied [1]. It is reported that reactive oxygen species (ROS) including ground-state atomic oxygen [O ($^3P_{j=0,1,2}$)], hydroxyl radicals (OH), metastable oxygen molecules ($^1O_2$), and superoxide anions (O_2^-) are effective in inactivating bacteria such as *E. coli* and *Bacillus*. Other reports are shown that the O or OH radical due to oxidation-decomposition of cell membrane is important factor for the inactivation. [2, 3] However, few studies have investigated the role of O (^3P_j) based on quantitative analysis of the gas phase.

We reported that the spores of *Penicillium digitatum* were rapidly inactivated by high-density non-equilibrium atmospheric pressure plasma (NEAPP) [4]. We investigated the inactivation effects of ozone and UV radiation. These results showed that the inactivation rate of this plasma was nearly one-thousandth of that of an ozonizer using the integrated number density of ozone measured by UV absorption spectroscopy and the contribution of UV radiation toward inactivation was not dominant for P. digitatum inactivation by NEAPP.

In this study, we investigated the inactivation of the spores by using an oxygen radical source that employs high-density atmospheric-pressure O_2/Ar plasma. The O (^3P_j) density was measured using VUV absorption spectroscopy. The inactivation efficiency of O (^3P_j) is discussed in terms of the atomic oxygen densities estimated by quantitative analysis of the gas phase. Furthermore, in order to investigate the change of the spores of *P. digitatum* treated by plasma, we also observed the spores by fluorescent microscopy.

2. Experiments

The radical source was developed based on the high-density NEAPP. The NEAPP generates high-density electrons of about 10^{16} cm^{-3}. [5, 6] The absolute density of O (^3P_j) was measured by VUV absorption spectroscopy using a microdischarge hollow-cathode lamp (MHCL). Measurements and exposure of radicals to spores were performed 10 mm downstream from the radical source. The inactivation efficiency of the spores was evaluated by colony-counting method.

Fluorescence image was observed by using an inverted microscope (IX 70 by Olympus) with

charge-coupled device camera (DP 72 by Olympus). 1,1'-Dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) was used as a vital fluorescence membrane dye [7]. Octadecyl group of DiI binds acyl group in the phospholipid of the cell membrane, so that the membrane is strained.

3. Results and Discussion

Figure 1 shows O (^3P_j) densities and *D* values, which represent indicator of inactivation, as a function of the $O_2/(Ar+O_2)$ flow rate ratio. The O densities were measured to be from $1.4x10^{14}$ to $1.5x10^{15}$ cm^{-3}. The O density increased with increasing $O_2/(Ar+O_2)$ mixture flow rate ratio up to 0.6% and it then decreased with increasing flow rate ratio. On the other hand, the *D* value decreased with increasing $O_2/(Ar+O_2)$ mixture flow rate ratio up to 0.6% and increased with increasing flow rate ratio. Since the *D* value is in inverse relation to the inactivation rate, O (^3P_j) is the dominant species in the inactivation.

In order to investigate the change of spores of *P. digitatum* by treatment of plasma, we observed the spores by fluorescent staining. Figure 2 shows fluorescence microscopic images of the spore stained by DiI. In the control, the membrane of the spore was stained by DiI (Fig. 2(a)). On the other hand, when the spore was treated by the plasma, the intracellular organelles of some spores emitted the fluorescent light by DiI (Fig. 2(b)). For the living cell, the DiI is not permeable because the membrane has selective permeability. Thus, it is suggested that the DiI penetrated the spore inside through the cell membrane. Therefore, it was speculated that the membrane was decomposed by the O or OH radicals produced from the plasma.

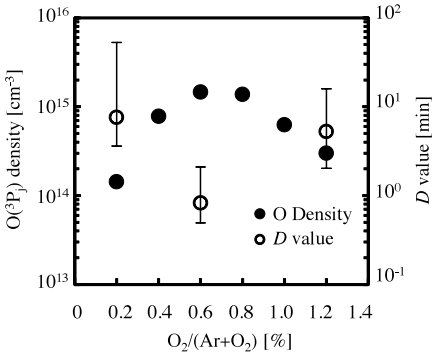
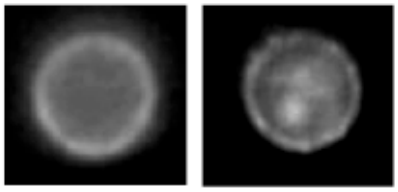


Fig. 1 O (^3P_j) densities and *D* values as a function of $O_2/(O_2+Ar)$ flow rate ratio.



(a) control (b) plasma treatment

Fig. 2 Fluorescent images of spores of *P. digitatum*.

4. Conclusion

We have inactivated *P. digitatum* spores using neutral ground-state atomic oxygen generated by an oxygen radical source employing a non-equilibrium atmospheric-pressure remote O_2/Ar plasma. The ground-state oxygen radical O (^3P_j) densities were measured using VUV absorption spectroscopy. The inactivation rate of *P. digitatum* spores inversely correlated to the O (^3P_j) densities. Accordingly, O (^3P_j) is one of the dominant species responsible for inactivating microorganisms. We also observed the spores of *P. digitatum* by using fluorescent microscopy using DiI in order to investigate the effect of oxidation. These results indicated that the cell membrane was destroyed by reactive species produced from the plasma due to the oxidation-decomposition.

5. Acknowledgment

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References

[1] A. Fidman, *Plasma Chemistry* (Cambridge University Press, New York, 2008).

[2] N. Hayashi, W. Guan, S. Tsutsui, T. Tomari and Y. Hanada, Jpn. J. Appl. Phys., **45** (2006) 8358.

[3] S. Perni, G. Shama, J. L. Hobman, P. A. Lund, C. J. Kershaw, G. A. Hidalgo-Arroyo, C. W. Penn, X. T. Deng, J. L. Walsh and M. G. Kong, Appl. Phys. Lett., **90** (2007) 073902.

[4] S. Iseki, T. Ohta, A. Aomatsu, M. Ito, H. Kano, Y. Higashijima and M. Mori, Appl. Phys. Lett., **96** (2010) 153704.

[5] M. Iwasaki, H. Inui, Y. Matsudaira, H. Kano, N. Yoshida, M. Ito and M. Hori, Appl. Phys. Lett. **92** (2008) 081503.

[6] H. Inui, K. Takeda, H. Kondo, K. Ishikawa, S. Makoto, H. Kano, N. Yoshida and M. Hori, Appl. Phys. Express, **3** (2010) 126101.

[7] M. G. Honig and R. I. Hume, J. Cell Biol., **103** (1986) 171.