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酸素ラジカル処理されたミドリカビ胞子の蛍光観察

Fluorescent observation of *Penicillium digitatum* spores treated by oxygen radicals

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Introduction: We reported that spores of *Penicillium digitatum* were rapidly inactivated by high-density non-equilibrium atmospheric pressure plasma (NEAPP).[1] Some papers have been reported that reactive oxygen species (ROS) are effective to inactivate microorganisms. We showed that ROS changed the function of cell membrane or cell wall without major morphological change in cell membranes, which leads to cell inactivation.[2] Furthermore, we found that ground-state atomic oxygen [$O(^3P_{j=0,1,2})$] is short life-time and the dominant factor responsible for inactivating *P. digitatum* spores using an atmospheric-pressure oxygen radical source, which only supplies neutral oxygen radicals.[3, 4] However, it is not clear how neutral oxygen radicals affect *P. digitatum* spores. In this study, we observed *P. digitatum* spores treated by oxygen radicals using a fluorescent dye.

Experimental: The chamber containing a radical source was purged with Ar gas to eliminate the influence of atmospheric gases. The spores were exposed to radicals 10, 15 and 20 mm downstream from the radical head, stained by 1,1'-dioctadecyl-3,3,Y,3'-tetramethylindocarbocyanine perchlorate (DiI), which has been used to investigate the structure and dynamics of cell membranes, and observed by confocal laser microscopy.

Results: Figure 1 shows the ratio of the number of intracellular stained spores by DiI at three different exposure distances. At a 10 mm distance, the ratio of the number of intracellular stained spores was 84% at 1.5 min treatment, and then decreased over 5-min treatment. On the other hand, at 15 and 20 mm distances, more treatment time was needed to increase the ratio of the number of intracellular stained spores. Considering the previously reported inactivation rate as a function of exposure distances, these results suggests that $O(^3P_j)$ affects the function of cell membrane or cell wall and oxidizes the intracellular organelles, which leads to the cell inactivation.

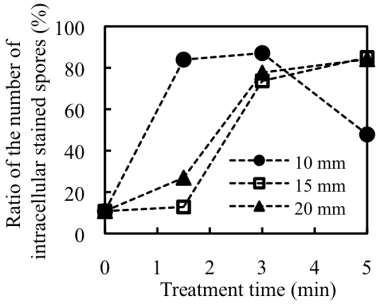

Fig. 1 Ratio of the number of intracellular stained spores as a function of radical treatment time at three different exposure distances.

[1] S. Iseki, et al., Appl. Phys. Lett., **96**, 153704 (2010).

[2] T. Ohta. et al., the 59th Spring Meeting of JSAP, 16p-B8-3 (2012).

[3] S. Iseki, et al., Appl. Phys. Express, **4**, 116201 (2011).

[4] H. Hashizume, et al., the 73rd Fall Meeting of JSAP, 13a-E1-35 (2012).