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# Plasma generated reactive oxygen species oxidized mold spores

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Non-equilibrium (also either non-thermal, cold) atmospheric-pressure plasmas (NEAPPs) have been studied to explore the potential of the use in biomedical, biological, and agriculture applications. [1] In many early studies revealed that the NEAPPs activate and / or inactivate microorganisms. However, as we know the complex composition of plasma species (a number ions, radicals, and photons), it is necessary to clarify which is major species to either activate or inactive the microorganisms. Thus, to simplify the plasma parameters, the studies can guide us an efficient use of the NEAPPs in target application.

In previous our studies, a couple of early work indicated neutral radicals are major plasma species e.g., the ground-state atomic oxygen O($^3P_j$) is the most effective oxygen reactive species to inactivate microorganisms. Iseki *et al* [2] investigated the effectiveness of the O($^3P_j$) in inactivating *Penicillium digitatum* (*P. digitatum*) spores. [3-4] Also, we investigated cell proliferation with nitric oxide (NO) irradiation. [5]

In the current study, we quantitatively elucidated the inactivation process of various mold spores, *P. digitatum*, *Aspergillus flavus* (*A. flavus*), and *Aspergillus niger* (*A. Niger*), using a fluorescent microscopic observation. In the experiment, a commercially available oxygen radical generator (Fuji Machine MFG. CO., LTD. FPA-10) was used and a fluorimetric hydrogen peroxide assay, diphenyl-1-Pyrenylphosphine (DPPP) assay, was used to detect reactive oxygen species inside of cells. Fig. 1(a) shows microscope images of *P. digitatum* spore with and without atomic oxygen radical treatment. Surprisingly, we see the intracellular fluorescence even though the treatment time was short at 1.5 min. This short treatment time give no physical damage in scanning electron microscopic investigation. (We do not show here.) This indicates somehow intracellular oxidized without physical damage of cell membrane.

Further investigation of *A. flavus* and *A. Niger*, we obtained similar results intracellular oxidization with atomic oxygen radical treatment. Treatment time was varied up to 10 min and we see the fluorescent intensity increased as increase of treatment time. Interestingly, the spores showed slightly different fluorescent intensity as increase of treatment time (dose, quantity of O($^3P_j$)). We will discuss further investigations of colony counting, fluorescent images and SEM results.

## References

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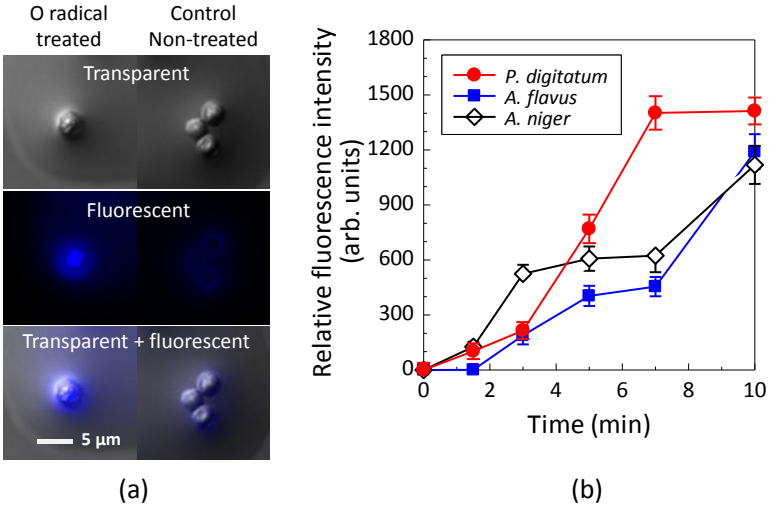


**Fig. 1** (a) shows microscope images of *Penicillium digitatum* spore with and without atomic oxygen radical treatment. The both fluorescent images clearly show a difference: fluoresce of inside of the cell was observed in case of with O radical treatment, while no fluoresce was observed without O radical treatment. (b) shows the fluorescent intensity of three mold spores, *P. digitatum*, *A. flavus*, and *A. Niger*. The intensities increased as a function of treatment time.