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Effects of the physical parameters of a microwave plasma jet on the inactivation of fungal spores

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ABSTRACT

In spite of their importance in human life, filamentous fungi have not been actively explored in the application of plasma to them. A plasma jet source at the atmospheric pressure was excited by 2.45 GHz microwaves and operated at low energy regime with an average power of 0.8 W–1.6 W. This microwave plasma was applied to examine fungal inactivation and find physical conditions of plasma (electrical power, pulse widths, and fed gases) at which the highest inhibition effects on fungal growth was achieved. Spore germination and hyphal growth of the fungus were dramatically decreased when oxygen was used in the plasma discharge, and this might be due to the elevation in the level of Oxygen (O) radical. The level of O radical in the plasma generated from Ar and oxygen was also enhanced by the increased power and pulse width. Hyphal growth of the fungus was more inhibited when greater power or longer pulse was applied. It appears that plasma effects were varied among different fungal species. Different levels of inhibition on spore germination and growth of three filamentous fungi, *Neurospora crassa*, *Fusarium graminearum*, and *Fusarium oxysporum* was observed.

1. Introduction

Microwave plasma at atmospheric pressure has been developed for surface modification, nano-particle production, gas abatement and biomedical applications including sterilization and detoxification [1–5]. Due to the low energy of ions that strike the electrodes and the low potential of plasma, microwave plasma is considered to be very useful for biological application. In addition, the size of device can be reduced even though microwave operating frequency is increased. In order to operate at the frequency of 2.45 GHz, a number of power modules and solid-state components developed for mobile communications systems are available at low cost in market.

The capability of microwave plasma to inactivate bacteria has been demonstrated in previous studies [1,3]. Compared to bacteria, filamentous fungi (producing hypha) have not been frequently explored to determine how they react to the plasma. Many filamentous fungi can cause serious problems to humans. They are prevalent everywhere and present a serious danger to immune-compromised people or economically important crop plants and animals [6,7]. Many fungal microbes live on human skin and form microbial flora [8]. Although the fungi in flora usually do not cause any problems, some fungi can cause infections under certain conditions. In order to control fungal diseases, various sterilization tools using heat, chemicals, and radiation have been utilized, but their efficiency and accompanying side effects when applied are controversial. Plasma seems to have more advantages in terms of environmental safety and human health. However, fungal microbes have different cell structures, all of which are more sophisticated than bacteria. Therefore, different plasma conditions may be required for efficient sterilization [9].

Although microwave plasma has shown a possibility as an efficient technique to replace traditional sterilization methods, it is still necessary to determine the physical parameters of plasma such as power, frequency, and fed gases by which the highest inactivation effects can be produced. Different effectiveness of plasma in the inactivation of microbes has been often observed between bacteria and fungi even though same plasma source is used [10–12]. Fungal microbes have different cell structures which are more sophisticated than bacteria and therefore plasma conditions may differently be applied to acquire same level of inactivation rate. Since the sterilization efficacy of plasma can be diverse depending on microbial species, fine tuning for plasma conditions (physical and electrical) might be needed for efficient application of plasma technology to the inactivation of fungi.

In this study, we have demonstrated inactivation of fungal spores by microwave plasma jet. Plasma jet was discharged at atmospheric pressure by using 2.45 GHz microwaves that are generated through the system consisted of a 2.45 GHz pulsed magnetron oscillator, a

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circulator, a directional coupler and a three-stub tuner. The microwave plasma-jet source was designed by using 3D High Frequency Structure Simulation (HFSS) code and operated at low energy regime with an average power of 0.8 W at frequency 2.45 GHz. Plasma conditions such as power, electric pulse, and fed gases were examined for the best inactivation effects using spores of three filamentous fungi, *Neurospora crassa*, *Fusarium graminearum and Fusarium oxysporum*. All fungal spores were inactivated within 60 s and proportion of reactive oxygen produced differently by using different fed gases was found to be a critical factor for the determination of sterilization efficiency.

2. Materials and methods

2.1. Filamentous fungi used in the study and culture condition

Filamentous fungi used in the study are *N. crassa* (wild type strain; ORS-SL6a), *F. graminearum* (wild type strain; PH-1), and *F. oxysporum* f. sp. *lycopersici* (race 1). *N. crassa* is a saprophytic filamentous fungus known as the bread mold, and *F. graminearum* and *F. oxysporum* are plant pathogenic fungi known as the head blight and the vascular wilt fungus, respectively. *N. crassa* was grown in Vogel's Minimal (VM) media and kept at 30 °C in the dark for 2 days and then at 25 °C in the light for at least 3 days. *F. graminearum* and *F. oxysporum* were cultured in Potato Dextrose Agar (PDA) plate at 30 °C in the dark.

2.2. Microwave plasma jet and plasma treatment to filamentous fungi

A microwave plasma jet source having coaxial structure was used in the study (Fig. 1). The gas was flown through the coaxial structure with flow rate of 3 slm (standard liter per minute). Gases used for plasma discharge were mixtures of Ar with oxygen (5:1), with nitrogen (5:1), or with air (5:1), and nitrogen gas. The microwave plasma jet was generated at input peak power of 100 W (pulse width: 40 μs, pulse period: 2500 μs, average power: 1.6 W).

Fungal spores exposed to plasma were harvested as follows. *N. crassa* was grown on VM-agar media in 100 ml flask at 30 °C. After grown for 1–2 weeks, 50 ml of sterile water was added in the culture flask and then mixed. Spores were collected through filtering suspensions. One microliter containing 5000 spores of *N. crassa* was placed on the center of VM-agar plate and then exposed to plasma for indicated time, using different gas mixtures. In order to get spores of *F. graminearum* and *F. oxysporum*, pieces of fungal hypha were inoculated into 100 ml of carboxymethylcellulose liquid media and incubated at room temperature with shaking for 3–4 days. Then, fungal spores were collected by filtering the liquid culture through 2 layers of miracloth (Calbiochem., USA). One microliter containing 1000 spores was placed on the center of VM-agar plate and then exposed to plasma. Fungal spores were placed 5 mm away from the end of jet device and exposed to microwave jet plasma using various power and gases ($Ar + O_2$, $Ar + Air$, $Ar + N_2$, N_2).

2.3. Analysis of optical emission spectrum and temperature

To analyze the components in plasma and sample temperature, optical emission spectrum and temperature of the area exposed to plasma were measured by using HR4000-UV-NIR OES spectrometer (Ocean Optics, USA) and Forward Looking InfraRed (FLIR) camera (BCAM SD infrared thermal imager), respectively. For temperature measurement, plasma was exposed to the center of VM-agar plate placed at 5 mm away from plasma device for 10 min. Right after plasma exposure, the center area of the agar plate was imaged by using FLIR.

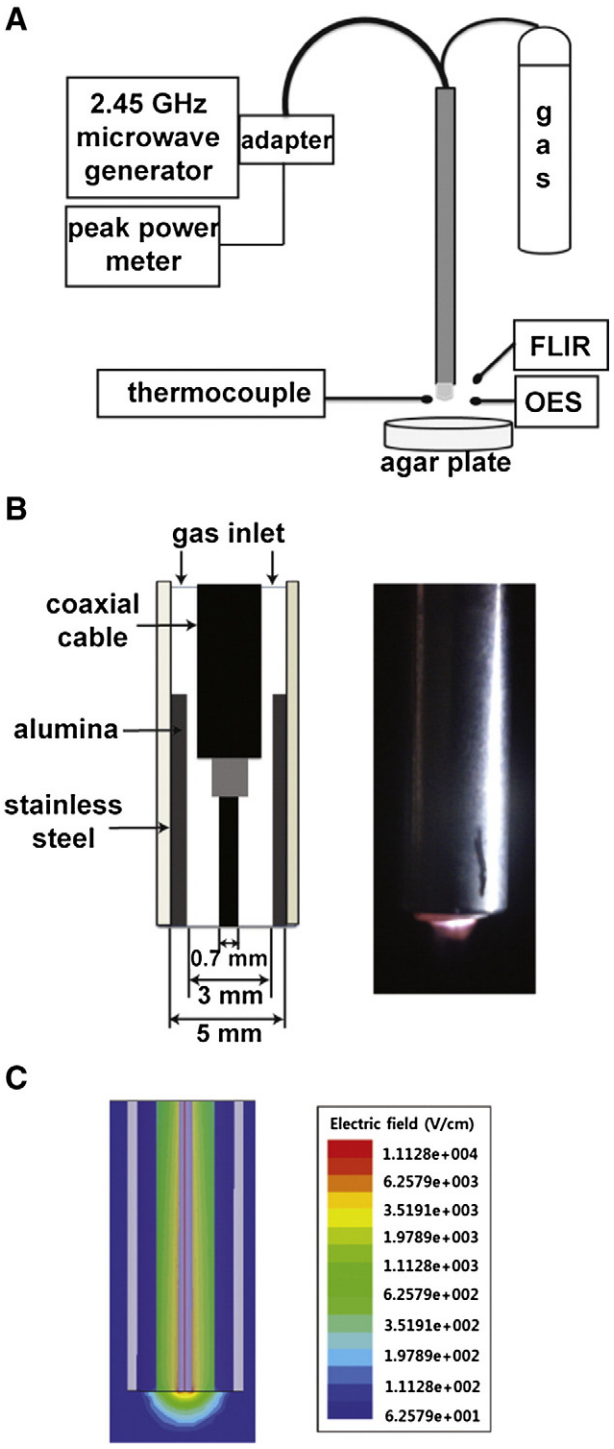

Fig. 1. Overview of microwave plasma jet device. A. Schematic view of a microwave generation system. B. Schematic view (left panel) and picture (right panel) of microwave plasma jet device. C. Electrical field generated in the vicinity of plasma source.

2.4. Measurement of fungal growth

The sterilization efficiency of the microwave plasma jet was assessed by examining germination and growth of fungal spores on the media plate. After exposure to plasma, fungal spores on VM-agar or PDA plates were incubated at 30 °C for 1 (for *N. crassa*) or 4–5 days (for *F. graminearum* and *F. oxysporum*). The diameter of fungal hyphal extension on the plate was measured, and the thickness and amount of fungal mycelia were visibly examined.