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The survival of micromycetes and yeasts under the low-temperature plasma generated in electrical discharge

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Abstract The fungicidal effect of low-temperature plasma generated by positive direct current discharge and its influence on the growth dynamics was evaluated on three micromycete species and yeast in water suspensions. The fungicidal effect was lower than analogous bactericidal effect and differs substantially among various fungal species. Together with the cidal effects, the slower growth of exposed fungal spores was observed.

Abbreviations

HEPA	High-efficiency particulate air filter
PBS	Phosphate-buffered saline
CFU	Colony forming unit(s)
YGCH	Yeast extract–glucose–chloramphenicol (medium)

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The low-temperature plasma generated in various types of electrical discharges is a partially ionized gas where most of energy is stored in the kinetic energy of electrons, whereas ions keep the room temperature. This ionized gas represents a cold mixture of free radicals and charged particles and does not increase the temperature of material applied on. There are numerous works describing the biological effects of this plasma, devoted mainly to the killing of prokaryotic bacteria (for review *see* Laroussi 2005; Scholtz et al. 2007a; Moreau et al. 2008) or various applications in human medicine (Fridman et al. 2008). Concerning fungi, only Akishev et al. (2008) mentioned the inactivation of *Aspergillus niger* and *Candida lipolytica* on agar surface after exposure with the plasma jet device. The possible application of plasma sterilization may be useful, e.g., for treatment of fruits' surface, preventing the mold overgrowth and bacterial putrefaction. This work presents the fungicidal effect of stabilized positive flashing corona discharge and its influence on the growth dynamics of three micromycete species and yeast in a water suspension.

Materials and methods

The low-temperature plasma was generated using the previously described simple apparatus of an open-air type (Julák et al. 2006). Briefly, the discharge burns on the point electrode represented by the tip of a syringe needle connected with a serial resistance of 20 MΩ to the positive pole of direct current high-voltage supply. The ground electrode was realized by the surface of water suspension of microorganisms connected with an immersed platinum wire to the negative pole of the supply. The discharge voltage was set to 9.7 kV and the distance between the tip of a needle and water surface was adjusted to 3 mm to get the

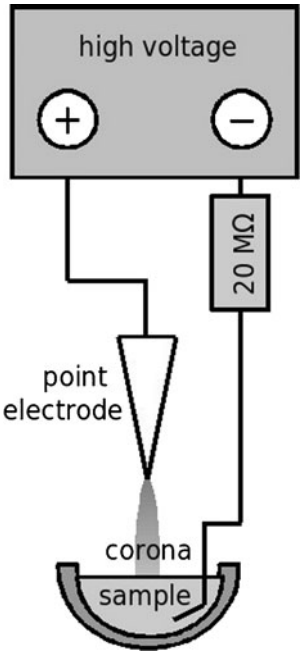

Fig. 1 Schematic experimental arrangement of inactivation of microbial suspensions

discharge current of 400 μA. All exposures were performed under laminar flow of HEPA-filtered air to prevent the airborne contamination; an air-conditioning of the laboratory controlled the ambient conditions (Fig. 1).

The conidia of fungal species *Aspergillus oryzae* (DBM 4002), *Cladosporium sphaerospermum* (DBM 4282), and *Penicilium crustosum* (DBM4159) and the cells of yeast *Candida albicans* were studied. They were cultivated for 5 days on YGCH medium (Bio-Rad, Czech Republic) at 20°C. The fungal conidia were harvested using a bacteriological loop and suspended in sterile PBS (pH 7.4). The suspensions of conidia and of the yeast cells were prepared immediately before the exposure to the discharge. The suspension (0.5 mL) was pipetted into the sterile wells of a dot plate and exposed to the discharge for various time intervals. Following the exposure, the content of each well was spread onto the surface of YGCH agar and, after the cultivation at 20°C, the number of surviving colonies (i.e., CFU) were counted.

Results and discussion

We observed that the number of surviving cells of yeast *C. albicans* decreased from 5×10^4 CFU/mL to zero after a 6-min exposure to the discharge. The total inactivation of *A. oryzae* spores does not occur even after a 30-min exposure only the reduction from 10^5 to 10^2 CFU/mL could be observed. In the case of *C. sphaerospermum*, the number of surviving cells was the same as the initial concentration of 10^5 CFU/mL for the first 10 min, then rapidly decreased up to a total inactivation within further 10 min. Also, the number of surviving cells of *P. crustosum* decreased during the first 15 min of exposure to one half of initial value (10^4 CFU/mL); during the next 5 min it decreased to the number of 40 CFU/mL and, finally, to total inactivation after next 5 min of exposure (Table 1).

The dynamics of micromycete growth after exposure with a sublethal dose of plasma was interesting: the surviving exposed fungal spores grow visibly slower than the nonexposed (Table 2). The appearance of the first colonies in exposed micromycetes was delayed 35 h for *Cladosporium*, 45 h for *Aspergillus*, and up to 65 h for *Penicilium*, as compared to nonexposed cultures. On the other hand, the time interval between appearance of growth and sporulation was shortened substantially differing with cultures grown from spores after discharge exposure. The number of fungal colonies on the agar surface was in each case ≈50 CFU/mL, which shows that the above differences could not be attributed to different growing conditions. Thus, it indicates that the exposed micromycetes sporulate faster than the nonexposed ones. In our previous work (Scholtz et al. 2010) with bacteria and yeast, similar effects were not observed.

Comparing these results with those obtained with bacteria, it may be concluded that bacteria are more susceptible than fungi to the low-temperature plasma. Using the less effective negative corona discharge (Scholtz et al. 2010), the complete inactivation of *Escherichia coli* and *Staphylococcus aureus* was achieved after 2 and 4 min of exposure, respectively. The bacterial spores of *Geobacillus stearothermophilus* were also more susceptible than the fungal spores (Scholtz et al. 2007b). The effectiveness of plasma generated by various discharges is apparent from the comparison of yeast inactivation, which occurred after 30 min under the negative corona but after 6 min under the positive discharge used in this work.

The low-temperature plasma generated by the positive corona discharge has the fungicidal effect differing for various species. Whereas the total inactivation of yeast happens in 6 min, the total inactivation of fungal spores needs 20–25 min of exposure for *C. sphaerospermum* and

Table 1 Survival of spores (expressed as colony forming unit per milliliter) exposed to positive corona discharge for 0–30 min

Fungus	0	5	10	15	20	25	30
C. sphaerospermum	1.5×10^6	9.0×10^4	1.1×10^5	10	0	0	0
P. crustosum	1.3×10^4	7.6×10^3	4.9×10^3	7.9×10^2	40	0	0
A. oryzae	1.4×10^5	7.0×10^3	2.8×10^4	2.9×10^4	2.5×10^4	1.0×10^3	1.1×10^2

Table 2 Growth and subsequent sporulation of spores exposed to positive corona discharge

Fungus	Treatment	Time to growth h	Time to sporulation, h	From growth to sporulation, h
C. sphaerospermum	Control[a]	45	80	35
	Exposed for 15 min	80	95	15
P. crustosum	Control[a]	50	115	65
	Exposed for 15 min	115	150	35
A. oryzae	Control[a]	70	115	45
	Exposed for 15 min	115	140	25

[a] Unexposed spores

P. crustosum. *A. oryzae* spores were not completely inactivated even after 30 min of exposure. The lower resistance of vegetative yeast cells is probably due to the unprotected cell membrane on comparison with the encapsulated fungal spores. The exposure to plasma retards the consecutive fungal growth: while nonexposed spores grow during 45 to 70 h, the exposed ones need 80 to 135 h. This stress also enhances the sporulation in cultures grown from exposed spores. The possible explanation of this effect is the stepwise mechanisms of spore inactivation with partially reversible disruptions in the structure of spore, which retards or inhibits the germination.

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