PLASMA JET IMPACT ON BACTERIAL CULTURES

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Abstract. Atmospheric cold plasma jet was used to test the effects on some microorganisms that could contaminate temperature-sensitive medical devices. The inactivation of the bacterial growth around plasma jet impact spot was measured and analyzed after 100 s exposure. Different behaviors of Gram-positive and Gram-negative bacteria were evidenced for the two plasma jet lengths: 2.5, and 3.5 cm, respectively. We attributed the results to the interaction of ultraviolet radiation (UV) generated by the plasma jet ozone with proteins found in high concentration in the cellular wall of Gram-negative strains compared to Gram-positive ones.

Key words: *Sarcina lutea, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa*, cold plasma jet, growth inhibition.

INTRODUCTION

During the past decades, researchers have paid considerable attention to the identification of appropriate methods for inactivating bacterial loading of medical objects while avoiding inconvenient heating of temperature sensitive materials such as polymers. UV radiation exposure was widely applied for surface sterilization as an alternative, non-invasive technique, where preservation of material features was needed [6]. However, this method has its limitations due to the reduced penetration depth of UV. An alternative method that combines the effects of UV rays and reactive oxygen species is represented by plasma jet

Received: September 2015;
in final form December 2015.

ROMANIAN J. BIOPHYS., Vol. 25, No. 4, P. 259–265, BUCHAREST, 2015

exposure. Due to its characteristics, plasma jet disinfection preserves the integrity of temperature sensitive medical utensils with microorganism loading [4, 8].

As known, atmospheric cold plasma is the result of an electric field applied between two electrodes that ionizes the discharge gas generating ions and free electrons. Because the discharge takes place in atmospheric air, it also produces high concentrations of oxygen and nitrogen species, high energy photons – mainly in UV range, all of these being continuously directed towards the target (by the electric field or/and gas feed), usually placed on the grounded electrode. All these plasma components can damage living microorganisms. Cold atmospheric plasma was found to be a quite efficient method to inactivate the exposed spores of Gram-positive *Bacillus subtilis* [16]. Plasma exposure succeeded in decolonization of both Gram-negative *Escherichia coli* and Gram-positive *Staphyloccocus aureus* [11] while significant inactivating *Candida albicans* yeast [12]. For the UV radiation produced in low-temperature electric discharges lethal effects were reported in the case of *Escherichia coli* [2] and *Bacillus atrophaeus* [5], due to the UV emissions as well as to UV generated ozone in synergistic action with OH radicals.

In this paper we present the results of cold plasma jet 100 s interaction with agar cultures of *Sarcina lutea, Bacillus cereus, Escherichia coli,* and *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

TEST GERMS

ATCC collection strains chosen as representative for Gram-positive bacteria for this study were *Sarcina lutea* ATCC 9341, and *Bacillus cereus* ATCC 14579, and *Pseudomonas aeruginosa* ATCC 9027, and *Escherichia coli* ATCC 35218 as Gram-negative bacteria, respectively. The original strain samples were preserved in lyophilized form until the experiment. Normal physiological sterile saline was used to prepare bacterial inoculums with a density of approximately $3 \cdot 10^8$ cells/mL adjusted through calibration curves. Equal volumes of 0.2 mL from each bacterial suspension were inoculated in 10 mL Mueller-Hinton (Oxoid) molten agar (pH 6.8). The agar cultures were grown in 100 mm diameter sterile plastic Petri plates at 37.0 ± 0.5 °C.

COLD PLASMA YIELDING AND SAMPLE TREATMENT

Helium gas flow of 0.15 L/min at atmospheric pressure was used to generate plasma jet by asymmetric dielectric barrier discharge (DBD); pulsed voltage of 9 kV peak to peak value, at 1.6 kHz frequency was applied, as described in [3, 13]. The agar cultures were exposed to plasma jet for two distances between the high voltage electrode and the grounded one: 2.5, and 3.5 cm, respectively. The Petri dishes were placed one at a time between the discharge electrodes, on a dielectric layer disposed over the plane ground electrode, and were exposed to the plasma jet for 100 s. Following, the Petri dishes were incubated for 24 h at 37.0 ± 0.5 °C, according to established microbiological protocols [9]. The control samples were inoculated from the same bulk vial and incubated in the same conditions except they were not exposed to the plasma jet.

The effects of plasma treatment were evidenced as circular zones of bacterial growth inhibition that were measured with 1 mm precision ruler. To assess the efficacy of the microorganism killing, the colony forming units (CFUs) within the growth inhibition zones were also counted.

Statistics

For each bacterial strain and for every exposure arrangement four repetitions were used to calculate the average values and standard deviations.

RESULTS

In Fig. 1, the response of *S. lutea* to the action of atmospheric plasma jet is presented. Each of the four exposures to 2.5 cm plasma jet resulted in quasi-equal circular areas with translucent appearance. Similar responses for 2.5 cm plasma jet were obtained for *B. cereus, E. coli* and *P. aeruginosa* (Fig. 2).

Fig. 1. *Sarcina lutea* response to 2.5 cm plasma jet after 100 s exposure.

The highest sensitivity to plasma jet action was revealed at the Gram-negative strains: *P. aeruginosa* exhibited the highest average value of the growth inhibition diameter, about 20 mm, followed by *E. coli* with an approximately 15 mm average diameter. Gram-positive bacteria have responded also to plasma impact but their sensitivity appeared to be lower: for *S. lutea* the average diameter was 11 mm, and for *B. cereus* only 10.5 mm. The standard deviation was of about 9%.

We evidenced remarkable difference between the responses of Gram-positive and Gram-negative strains for the exposures to the 3.5 cm plasma jet (Fig. 3). Thus, *S. lutea* and *B. cereus* were resistant to plasma impact – with zeroed diameter of growth inhibition, i.e. no lethal effect could be revealed on the corresponding agar cultures. On the contrary, *E. coli* and *P. aeruginosa* remained vulnerable to plasma impact, the growth inhibition areas having average diameters of approximately 11 mm each.

The counting of CFUs within the inhibition zones revealed an average percentage of surviving bacteria between 5 and 23% in the case of *E. coli* and *B. cereus* for 2.5 cm, and between 4 and 34% in the case of *E. coli* in the case of *P. aeruginosa* for 3.5 cm. Therefore we can assess the inactivation efficiency of plasma jet as ranging between 95% and 77% for 2.5 cm plasma jet, and between 96% and 66% for 3.5 cm plasma jet, respectively.

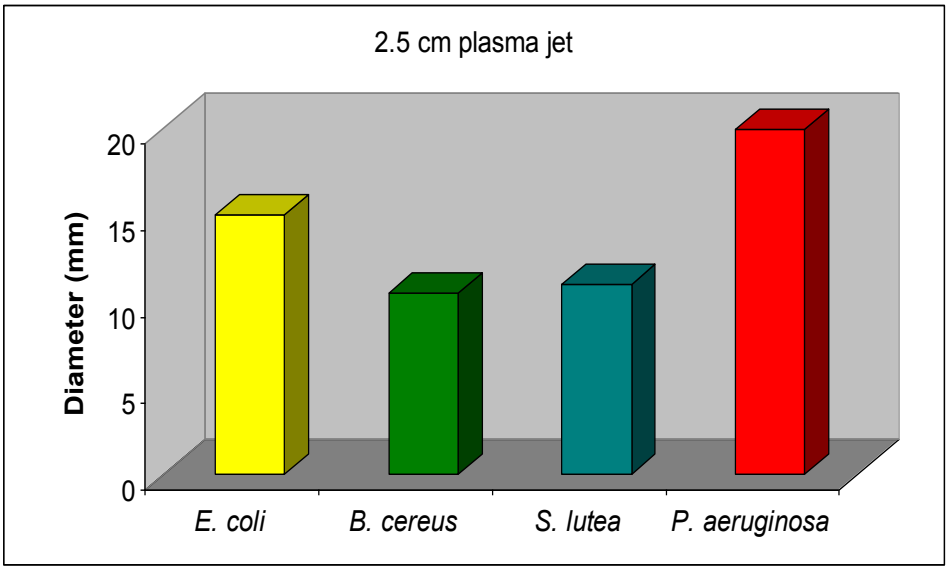

Fig. 2. The size of growth inhibition areas for 2.5 cm plasma jet after 100 s exposure.

We attribute the smaller inhibition zones for 3.5 cm compared to 2.5 cm plasma jet exposures to the diminished physical impact of corpuscular radiation (ions, electrons, and free radicals mainly reactive oxygen species) caused by the higher attenuation in the atmospheric air in the case of the longer trajectory. This could explain the reduced responses of all analyzed germs for the two Gram-positive strains.

At the same time, for the longer jet the higher content of UV generated ozone [1] can be related to the behavior of Gram-negative bacteria. They remained sensitive to 3.5 cm plasma jet length probably because of their higher content of proteins in the cellular wall [10] that might be affected by the interaction with the ozone. Some authors reported the high sensitivity of Gram negative bacteria in aqueous media ozone [14], some others assumed that ozone reacts more readily with proteins than lipids and further [7]. Based on this hypothesis, the higher content of proteins from the cellular wall of the Gram-negative bacteria more sensitive to ozone as compared to the Gram-positive microorganisms [15] could explain our findings.

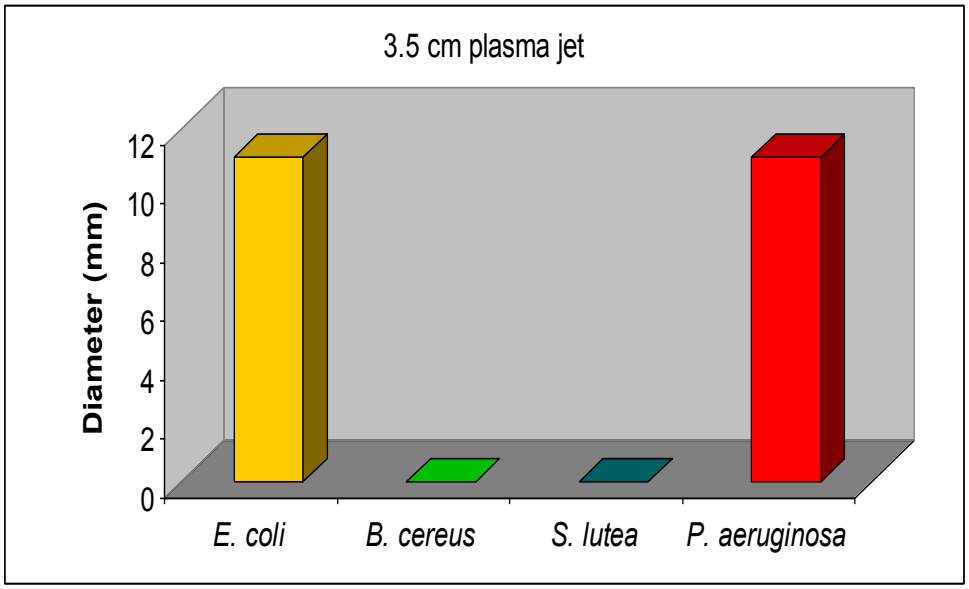

Fig. 3. The size of growth inhibition areas for 3.5 cm plasma jet at 100 s exposure time.

However, the differentiate response of the microorganism to plasma jet length seems to be more complex, so that the above hypothesis could not provide a definite criterion to generalize the behavior of Gram-positive *versus* Gram-negative germs. In similar experiments performed on other microorganisms [13] we found that only Gram negative yeast (*C. albicans*) responded with smaller growth

inhibition area for the longer plasma jet – for the same exposure time of 100 s. Instead, some Gram positive bacteria strains (*S. aureus* and *B. subtilis*) exhibited higher sensitivity to longer plasma jet which suggests that bacterial species could react differently even in the same large category established by Gram-classification. Moreover, we could say that, in the frame of the same species, different strains could be characterized by peculiar biological features that could reflect in the sensitivity to cold plasma exposure; indeed *E. coli* bacteria, strain ATCC 25922, used in the experiment described in [13], gave an opposite response compared to *E. coli* strain ATCC 35218 presented in this study. To resume, in these experiments we found that, for the chosen microorganism strains, the highest intensity of atmospheric plasma bio-effects was evidenced for the Gram-negative strains – otherwise known for their complex cellular wall conferring resistance against chemical treatments (drugs or disinfection chemicals). The results recommend cold plasma jet as an efficient, chemical and liquid-free method for the inactivation of those bacteria that can usually be killed with certain difficulties at ambient temperature. Nevertheless, the influence of plasma jet length on bacterial cells seems to necessitate further investigations while the results interpretation needs to take into account also the particularities of the species and strains.

CONCLUSION

We evidenced the inactivation efficacy of cold plasma jet on *S. lutea*, *B. cereus, E. coli*, and *P. aeruginosa* in a specific laboratory arrangement. The 2.5 cm plasma jet successfully inactivated microorganisms from all tested strains, due to the synergistic impact of corpuscular components – as ions and free radicals, mainly reactive oxygen species, and UV photons able to generate ozone. Microbial killing efficacy ranged between 95% and 77% for *E. coli* and *B. cereus*, respectively, as found from CFUs counting. The impact of 3.5 cm plasma jet – containing higher UV generated ozone – resulted in a killing efficacy of 96% for *E. coli*, and 66% for *P. aeruginosa,* respectively. Further experiments are planned to optimize the disinfection procedure based on these results.

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