INFLUENCE OF COLD ATMOSPHERIC PLASMA OF MICRODISCHARGE ON FUNGAL MYCELIUM AND SPORES GROWING

Yu. Veremii[1], I. Andriiash[1], N. Tsvyd[2], V. Chernyak[1], M. Sukhomlyn[2], Eu. Martysh[1]

[1]Taras Shevchenko National University of Kyiv, Faculty of Radio Physics, Electronics and Computer Systems, Kyiv, Ukraine;
[2]Taras Shevchenko National University of Kyiv, Educational and Scientific Center "Institute of Biology and Medicine", Kyiv, Ukraine

E-mail: veremii@i.ua

The effect of cold plasma microdischarge on the development of spores and mycelium of fungi was investigated. The influence was investigated on Penicilium sp. The results of cold-plasma spores' treatment showed that it could damage their outer shell and slow the colony growth, as well as stimulate germination of spores.

PACS: 52.77.−j

INTRODUCTION

Species of *Penicillium* are ubiquitous soil fungi, commonly present wherever organic material is available. Saprophytic species of *Penicillium* live mainly on organic biodegradable substances. Commonly known in America as molds, they are among the main causes of food spoilage [1]. Many species produce highly toxic mycotoxins [2]. Some *Penicillium* species affect the fruits and bulbs of plants, apples, pears, and citrus fruits [3, 4]. Some species are known to be pathogenic to animals and mosquitoes [5].

Cold plasma discharge application to the microorganisms is among the developing methods of microorganism inactivation and elimination. Cold plasma discharge methods possess a number of significant advantages, such as safe and controlled plasma application to the organisms. Due to the recent availability of cold plasma discharge sources that are spatially uniform and highly controlled, their application in atmosphere pressure conditions has become a reality in the realm of medicine, namely in microorganism inactivation and elimination.

Current methods of inactivation and elimination of microorganisms and their spores possess a number of disadvantages: high cost, difficulties with usage of the method among the potential users, formation of remainders on the processed surface, formation of disinfection products, change in properties of the surface and, finally, development of resistant strains of microorganisms.

As soon as fungal microorganisms play important role in agriculture, medicine and pharmacy, our research may establish a basis for further investigation of cold plasma discharge influence on fungal microorganisms and its application in various realms of industry, agriculture and medicine.

METHODOLOGY AND EXPERIMENTAL SET-UP

A pure culture of *Penicillium sp*. obtained from the Cultures Collection of Fungi (FCKU) of Educational and Scientific Centre "Institute of Biology and Medicine" Taras Shevchenko Kyiv National University. The culture was cultivated on potato-glucose agar (PGA) medium for spores formation. The *Penicillium* spore put into Petri dish with PGA medium and incubated at 23…25°C temperature. Part of the experimental samples was process with plasma discharge on the day of *Penicillium* spore inoculation, the rest – a day after inoculation. The control samples were Petri dishes with *Penicillium* spores that were not process by the plasma discharge. The plasma discharge was act on *Penicillium* spores within 3 min at different distances (1, 3, and 5 cm) as to the surface PGA medium. The influence of plasma discharge on spores germination was determined by the appearance of a growth retardation zone in *Penicillium* colony on the next day.

The scheme of the experimental set-up is shown in Fig. 1. The processing of selected objects was carried out by a plasma microdischarge torch [6].

The construction of the microdischarge generator has an axially symmetric system in which the internal electrode has water cooling (1). The indicated electrode is separated from the body of the system by a dielectric tube (4). Tangentially, the working gas (air) is introduced to the ground surface through the opening (2), which is blown out of the opening d = 1.5 mm for the airflow G = 1 l/min. Different electrodes (3) (internal in the form of a wire with a diameter of 1 mm and an external flat electrode with a thickness of 1 mm) are located at a distance of 1 mm apart from one another.

The operating current and voltage were I ≈ 15 mA, U ≈ 5…10 kV, so, supplied power was P ≈ 7.5…15 W. The temperature at each of heights of the treatment was measured using the thermocouple and was at the height of 1 cm – 100…120 °C, at the height of 3 cm – 60…70 °C and at the height of 5 cm – 30…35 °C. The distance to the specimen was adjusted by moving the cup on the tripod with the adjusting screw.

Microscopy was carried out using Microscopy PrimoStar light microscope and was performed with the

ScienseLabDCM 520 digital camera and the Axiovision 4.3.7 image-processing program.

RESULTS OF PLASMA TREATMENT

In the experimental samples that processed by plasma discharge at a distance of 1 cm was formed clearly expressed growth retardation zone (Fig. 2, B). Two days after inoculation around the growth retardation zone appeared young colonies that formed due to germination of intact spores.

In the Petri dishes that processed by plasma discharge at a distance of 3 cm has less perceptible growth retardation zone than in previous samples (Fig. 2, C). The samples that processed by plasma discharge at a distance of 5 cm had not a zone of growth retardation of *Penicillium* colonies (Fig. 2, D).

The formation of branching hyphae that formed conidial sporulation was noted by microscopy into control samples (Fig. 3,A). The spores size was about 4 μm into control dishes but in samples which processed by plasma discharge at a distance of 1 cm had size 6 μm (Fig. 3,B). The plasma discharge at a distance of 3 cm led to partial damage to the spores *Penicillium sp*. (Fig. 3,C). The spores on which the plasma discharge was applied at a distance of 5 cm formed large number hyphae of mycelium *Penicilium sp*. (Fig. 3,D).

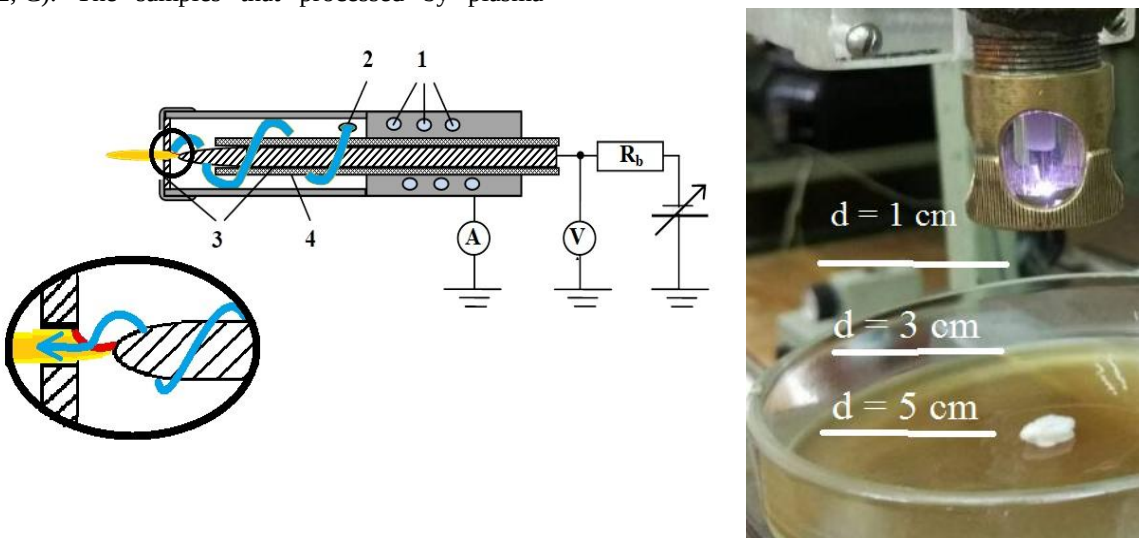

Fig. 1. Experimental set-up

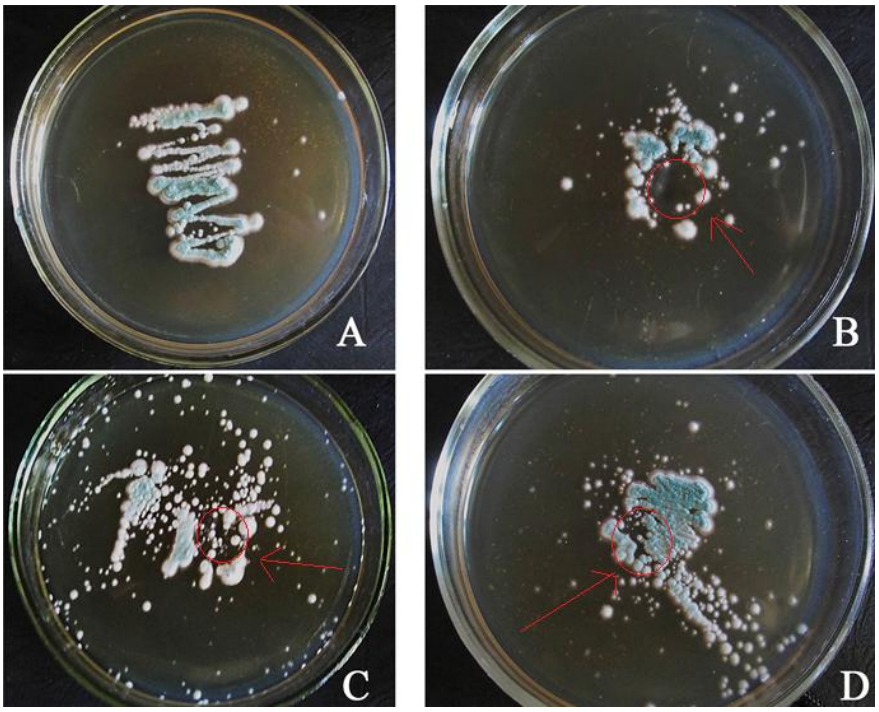

Fig. 2. The growth retardation zone of Penicilium sp. when processing plasma was on the day of sowing: A – control; B – plasma processing at a distance of 1 cm (90 ° C); C – Plasma treatment at a distance of 3 cm (70…60 °C); D – Plasma treatment at a distance of 5 cm (30 °C)

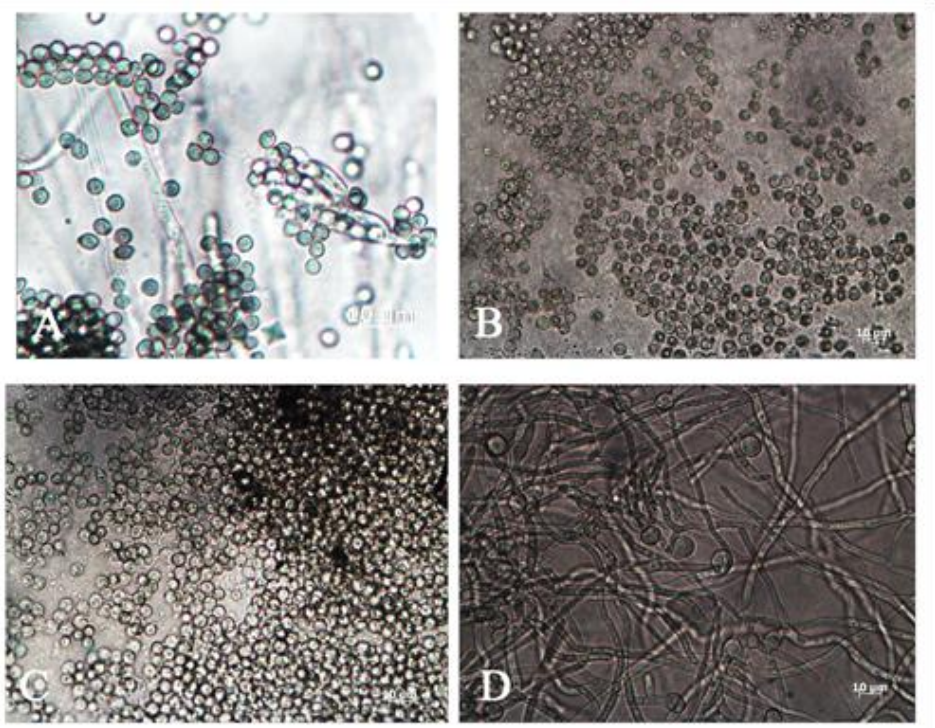

Fig. 3. Microphotography of Penicilium sp. spores: A – control; B – plasma processing at a distance of 1 cm; C – plasma treatment at a distance of 3 cm; D – plasma processing at a distance of 5 cm

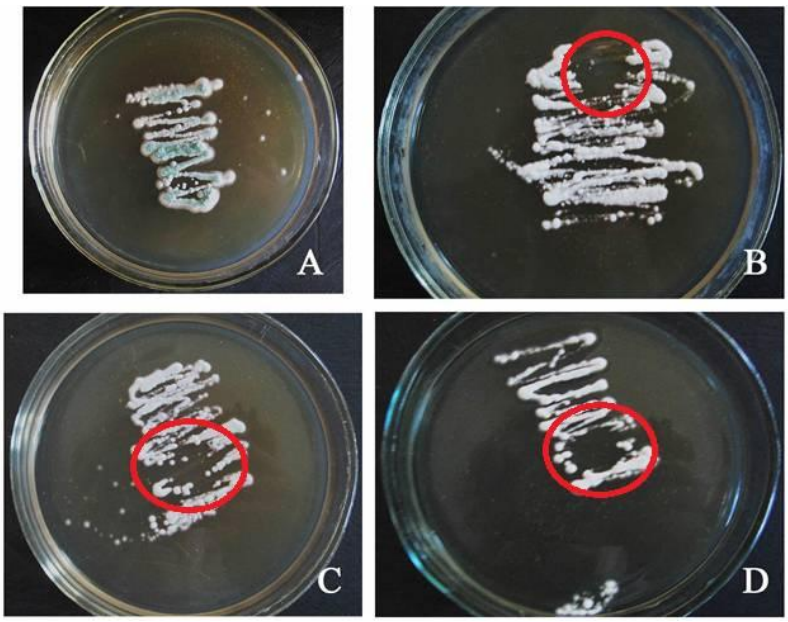

Fig. 4. The growth retardation zone of Penicilium sp. when processing plasma was on the day of sowing: A – control; B – plasma processing at a distance of 1 cm (90 °C); C – plasma treatment at a distance of 3 cm (70…60 °C); D – plasma treatment at a distance of 5 cm (30 °C)

All samples that process with plasma discharge a day after inoculation there was clearly expressed growth retardation zone of *Penicillium* colony (Fig. 4).

Also, these samples have damages on spore's germination stage which were detected by microscopy. This was expressed in partial deformation and actually an absence cellular spore membrane that differs in control samples. (Fig. 5). Compared to control sample A, sample B (1 cm) has 80 % of damaged hyphae, sample C (3 cm) – 60 % of damaged hyphae, and sample D (5 cm) – 30 %.

The cold plasma discharge processing of Petri dishes with *Penicillum sp.* resulted in growth lag of the colonies. At the same time, the size of the spores in processed colonies appeared to be damaged with the size 2 μm bigger than in a control dish. We also found that *Penicillum sp.* spores were more sensitive to plasma processing on the second day after inoculation. This means that *Penicillum sp.* may be more sensitive to plasma processing on the stage of germination.

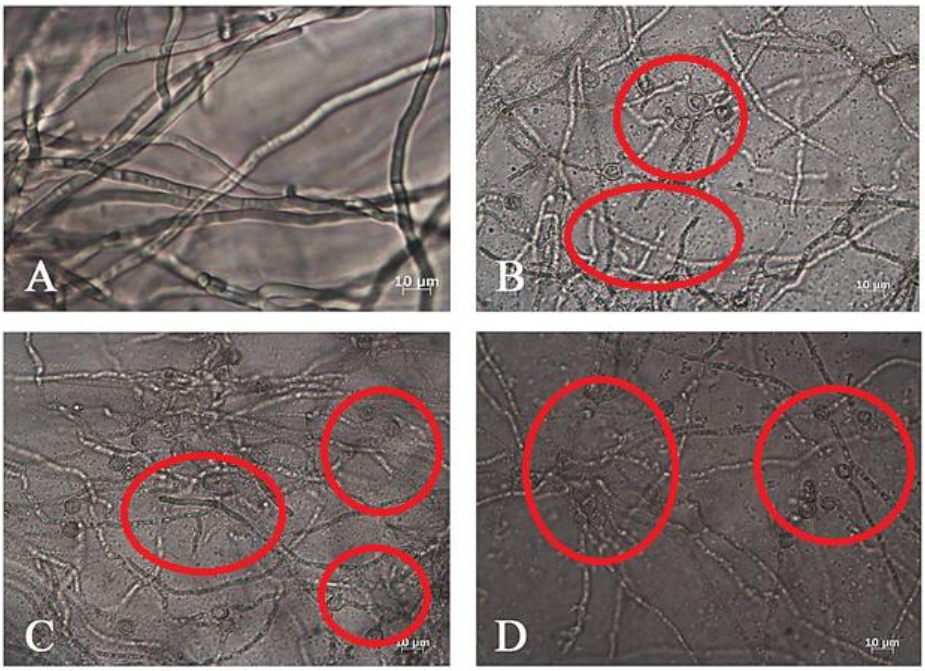

Fig. 5 Microphotography of Penicilium sp. hyphaes: A – control; B – plasma processing at a distance of 1 cm; C – plasma treatment at a distance of 3 cm; D – plasma processing at a distance of 5 cm

CONCLUSIONS

Cold plasma discharge processing of Petri dishes with *Penicillum sp.* resulted in growth lag of the colonies. At the same time, the size of the spores in processed colonies appeared to be damaged with the size 2 μm bigger than in a control dish (~ on 30 %). We also found that *Penicillum sp.* spores were more sensitive to plasma processing on the second day after sowing. This means that *Penicillum sp.* may be more sensitive to plasma processing on the stage of germination.

REFERENCES

1. R.A. Samson, K.A. Seifert, A.F. Kuijpers, J.A. Houbraken, J.C. Frisvad. Phylogenetic analysis of Penicillium subgenus Pencillium using partial beta-tubulin sequences // *Studies in Mycology*. 2004, v. 49, p. 175-200.

2. J.I. Pitt, J.C. Basílico, M.L. Abarca, C. López. Mycotoxins and toxigenic fungi // *Medical Mycology*. 2000, v. 38 (Suppl 1), p. 41-46.

3. B. Balgrie. *Taints and Off-flavours in Food*. CRC Press, 2013.

4. J.G. Valdez, M.A. Makuch, A.F. Ordovini, et al. First report of Penicillium allii as a field pathogen of garlic (Allium sativum) // *Plant Patholog.* 2006, v. 55 (4), p. 583.

5. G.L. Costa, A.M. Moraes, P.C. Oliveira. Pathogenic action of Penicillium species on mosquito vectors of human tropical diseases // *Journal of Basic Microbiology*. 1998, v. 38 (5-6), p. 337-341.

6. Eu.V. Martush et al. Investigation of a microdischarge system with the vortex gas flow // *Ukr. J. Phys.* 2016, v. 1, p. 960-962.

Article received 22.12.2018

ВЛИЯНИЕ ХОЛОДНОЙ АТМОСФЕРНОЙ ПЛАЗМЫ МИКРОРАЗРЯДА НА РАЗВИТИЕ МИЦЕЛИЯ И СПОР ГРИБОВ

Ю. Веремий, И. Андрияш, Н. Цвыд, В. Черняк, М. Сухомлын, Е. Мартыш

Исследовано влияние холодной плазмы микроразряда на развитие спор и мицелия грибов. Влияние исследовали на образцах Penicilium sp. Результаты обработки спор холодной плазмой показали, что она может повредить их внешнюю оболочку и замедлить рост колонии, а также стимулировать прорастание спор.

ВПЛИВ ХОЛОДНОЇ АТМОСФЕРНОЇ ПЛАЗМИ МІКРОРОЗРЯДУ НА РОЗВИТОК МІЦЕЛІЮ І СПОР ГРИБІВ

Ю. Веремій, І. Андріяш, Н. Цвид, В. Черняк, М. Сухомлин, Є. Мартиш

Досліджено вплив холодної плазми мікророзряду на розвиток спор і міцелію грибів. Вплив досліджували на зразках Penicilium sp. Результати обробки спор холодною плазмою показали, що вона може пошкодити їхню зовнішню оболонку і уповільнити розростання колонії, а також стимулювати проростання спор.