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STUDYING OF FUNGAL SPORES (ASPERGILLUS NIGER) TREATED WITH NOVEL PLANT-DERIVED ANTIMICROBIAL PEPTIDE NIGELLIN-1A ISOLATED FROM BLACK CUMIN (NIGELLA SATIVA) SEEDS*

Keywords: antifungal peptides, cellular mode of action, plant pathogenic fungi, Aspergillus niger, Nigella sativa.

Defense peptide are found in all plants discovered and represent one of the main protective line against biotic environmental stress factors like pathogenic microorganisms (bacteria, fungi, viruses, viroids) and pests (insect, mites and nematodes). Microscopy techniques are a powerful tool allowed to qualitatively and quantitatively evaluate effects of the peptide action at cellular and sub-cellular levels. In this work, we describe biological action of the novel antimicrobial peptide derived from black cumin seeds named as nigellin-1a. This peptide consisted of 38 amino acid residues and characterized by three disulfide bonds in molecule which is allowed to classify them as the novel member of hairpin-like defense peptides.

For realization of this microscopy investigation of features of peptide's action, the spores of pathogenic fungus Aspergillus niger were incubated with nigellin-1a, taken at various concentrations. The samples obtained were investigated using the fluorescent dyes SYTO 9 and propidium iodide (PI) provided as LIVA/DEAD cells viability assay kit (Invitrogen, USA) allowed evaluating the structural integrity of spores.

In the normal state fungus spores are impermeable to the PI. Incubation with peptide led to a pronounced disturbance of spore shells (Fig. 1 a) allowing to penetration of PI. So, after 3 hour of incubation with nigellin-1a, increase in permeability was recorded (Fig 1 d), that was visualized as an intracellular glow. Confirmation occurred morphological changes were given by an atomic force microscopy. After incubation for 6 hour the cells were visualized with signs of ejection of intracellular contents into the environment (Fig. 1 b). Also changes in the dimensional parameters were observed. The cellular volume in normal to be 31.62 ± 0.3 μm³, whereas the peptide-treated cells had a volume of 9.72 ± 1.0 μm³.

Thus is possible to state that the nigellin-1a has a membrane-acting and destructive mode of action on fungal spores.

*This work was supported by the Russian Science Foundation (grant № 18-74-10073).
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Fig. 1. The microscopy images of A. niger spores treated with nigellin-la (a, b, d). Bright-field microscopy (a) and fluorescence of treated spores stained with LIVE/DEAD kit (b). AFM-images of A. niger spores in normal (c) state and treated with nigellin-la (d). Bar scale – 20 μm (a, b); 2 μm (c); 1 μm (d).

The arrows indicate the lysed spores.