SUMMARY

The components of the fungal cell wall play a key role in the interaction between the pathogen and the host organism. Some of them play a role of the molecular structures called pathogen associated molecular patterns (PAMPs), which are recognized by the appropriate pattern recognition receptors (PRRs) of the host. One of the polysaccharide components of the cell wall of many fungal species pathogenic for humans, including *Aspergillus*, *Histoplasma*, *Blastomyces*, *Cryptococcus*, *Pneumocystis* and *Coccidioides*, is α-1,3-glucan. Its role in inducing the immune response is not entirely clear. Alpha-1,3-glucan is described as a molecule whose presence in the fungal cell wall protects against recognition by the host's immune system. This process occurs by masking the molecules considered as PAMPs which are located in the deeper cell wall layers. However, few scientific reports point to the immunomodulatory nature of this polysaccharide.

Due to the fact that the mechanisms of innate immunity of mammals and insects show a high degree of structural and functional similarity, insect-model organisms are used in the research on fungal pathogens, as well as on the immune response to their cell wall components. One of them is the greater wax moth *Galleria mellonella*, used in studies of fungal virulence, among others of *Aspergillus fumigatus*, *Histoplasma capsulatum*, *Cryptococcus neoformans*, *Blastomyces dermatitidis*, as well as in the study of the immune response to the components of their cell wall, e.g. β-1,3-glucan.

Due to the lack of literature data on the role of α-1,3-glucan in the induction of insect immune responses, this work investigates the immune responses of *G. mellonella* to this polysaccharide component of the *Aspergillus niger* cell wall. The aim of the study was to check if *A. niger* α-1,3-glucan is recognized by the *G. mellonella* immune system, and whether its presence in a hemocoel activates cellular response and mechanisms of humoral response, such as activation of the phenoloxidase system and antimicrobial peptides' synthesis.

G. mellonella haemolymph proteins that interacted with *A. niger* α-1,3-glucan were identified after sequencing by Edman degradation, as well as by mass spectrometry and immunoblotting with selected specific antibodies. In order to determine if the immune system of larvae reacts to the administration of α-1,3-glucan, the level of antimicrobial activity in haemolymph was assessed by radial diffusion assay against *Escherichia coli* and *A. niger*. Antimicrobial activity was confirmed by bioautography. To demonstrate differences in *G. mellonella* haemolymph proteins-peptides profiles after immunization with α-1,3-glucan, one-

dimensional and two-dimensional electrophoretic separations of proteins and peptides under denaturing conditions (SDS-PAGE, 2D IEF/SDS-PAGE) were performed. Antimicrobial peptides appearing in *G. mellonella* haemolymph in response to *A. niger* α-1,3-glucan were identified after sequencing by Edman degradation, and changes in their level in haemolymph were determined after resolution using RP-HPLC. Induction of gene expression of selected defence peptides (galiomycin, gallerimycin, cecropin, insect inhibitor of metalloproteinases) in *G. mellonella* fat body after administration of α-1,3-glucan was analyzed using Real-Time qPCR. The involvement of proteins constitutively present in haemolymph, lysozyme and apolipophorin III (apoLp-III), was also assessed in response to *A. niger* α-1,3-glucan. The effect of immunization with α-1,3-glucan on the apoLp-III level in *G. mellonella* haemolymph was determined by immunoblotting, and the location of apoLp-III in haemocytes was assessed by immunocytochemistry using specific antibodies directed against this protein. The induction of a cellular response to *A. niger* α-1,3-glucan was determined by the analysis of changes in the haemocytogram of *G. mellonella* larvae, and the analysis of the nodulation process.

Studies carried out in this work have shown that *A. niger* α-1,3-glucan is recognized by the immune system of *G. mellonella* larvae, probably mediated by β-1,3-glucan recognition proteins βGRPs receptors that bind to this polysaccharide. Among the *G. mellonella* haemolymph proteins that interacted with *A. niger* α-1,3-glucan, were also identified the following: apolipophorin I, apolipophorin II, prophenoloxidase, phenoloxidase activating factor, hexamerin, arylphorin and serine protease similar to peptidases from other representatives of the Lepidoptera order. It has been shown that in the reaction to the recognition of *A. niger* α-1,3-glucan, the expression of defence peptide genes in the *G. mellonella* fat body was induced, in particular a significant increase in the expression of antifungal peptide genes, galiomycin and gallerimycin, was detected. The consequence of this was the increase in the level of antifungal peptides in haemolymph, galiomycin among others, leading to appearance of anti-*A. niger* activity in *G. mellonella* haemolymph. It has been shown that the activity of the phenoloxidase system, one of the first mechanisms triggered in response to infection, has been inhibited in a short time after immunization with *A. niger* α-1,3-glucan It probably happened due to binding of prophenoloxidase and serine proteases involved in its activation to the polysaccharide. The observed changes in apoLp-III levels in *G. mellonella* haemolymph after administration of α-1,3-glucan indicate its contribution in response to this component of the fungal cell wall, although this protein is unlikely to be involved in signalling the presence of α-1,3-glucan to the haemocytes, since apoLp-III binding to this polysaccharide has not been demonstrated. The activation of the cellular

response of *G. mellonella* after immunization with *A. niger* α-1,3-glucan was evidenced by changes in the total haemocyte count (THC) and differential haemocyte count (DHC) as well as by the formation of nodules.

The results obtained in this study indicate that *A. niger* α-1,3-glucan is recognized by the immune system of *G. mellonella* larvae, and in response to this fungal cell wall component, a particularly an antifungal response is activated. Thus, α-1,3-glucan may play the role of a fungal PAMP molecule. On the other hand, administration of *A. niger* α-1,3-glucan caused a temporary inhibition of the immune response of the insect, including phenoloxidase activity, which may indicate that it plays the role of the *A. niger* virulence factor.