Antifungal activity of galectin-3 against black *Aspergilli*

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Objectives. The *Aspergillus* section *Nigri* comprises several species widely distributed in the environment. Apart from their use in food production and biotechnology, many species are plant pathogens, mycotoxin (ochratoxin A and fumonisins) producers, and causative agents of respiratory infections in immunocompromised patients as well as otomycosis and keratomycosis in tropical and subtropical regions [1].

Galectin-3 (Gal-3) is a pleiotropic protein that primarily binds to lactose and it is involved in a variety of cellular processes including interaction with pathogens, immune response and cell death. Several studies indicated possible antimicrobial action of Gal-3 against bacteria and yeasts but antifungal activity against filamentous fungi such as *Aspergilli* is not known [2].

The aim of this study was to test antifungal activity of Gal-3 in comparison to amphotericin B (AMB) against *A. brasiliensis* ATCC 16404 and 5 airborne species of black *Aspergilli* (*A. piperis*, *A. tubingensis*, *A. welwitchiae*, *A. niger*, *A. luchuensis*) from microbial collection of University of Zagreb, Faculty of pharmacy and biochemistry, Department of microbiology.

Methods. The *Escherichia coli* strain BL21(DE3) star transformed with plasmid pET-3c containing Gal-3 coding sequence and ampicillin resistance, was a kind gift from Dr Hakon Leffler. Expression of Gal-3 in these bacteria was induced by incubation (3 hours) with 0.5 mM IPTG. rh-Gal-3 was isolated from the lysate of induced bacteria on lactosyl-Sepharose column. Lactose was removed from rh-Gal-3 fractions by dialysis and SDS-PAGE and Western-blot analysis was used to confirm successful production and purification of rh-Gal-3.

A modified broth dilution method for susceptibility testing against *Aspergillus* (EUCAST-AST-ASPERGILLUS) was employed in this study [4]. Dilutions of AMB (0.015-16 µg/mL) and Gal-3 (8-520 µg/mL) in RPMI 1640 with 2% glucose inoculated with conidial suspension of black *Aspergilli* ($1-2.5 \times 10^5$ CFU/mL) were incubated for 48 h at 35°C. Upon incubation MTS reagent was added and plates were incubated for 3 h at 35°C. The absorbance was measured using a microplate reader (LabSystem iEMS, type 1404) at a wavelength of 492 nm. All tests were performed in triplicate and viability of *Aspergilli* was expressed as percentage of control (untreated *Aspergilli*).

Fig. 1. Viability of black *Aspergilli* after the treatment with Gal-3 and LPS.

Results. According to minimum inhibitory concentration (MIC) of AMB, *A. brasiliensis* ATCC 16404 (1 µg/mL) followed by *A. piperis* (0.5 µg/mL) were the most resistant black *Aspergilli* (Table 1). MIC of AMB for the rest of tested strains was 0.25 µg/mL. Opposite to AMB, Gal-3 was efficient only against *A. brasiliensis* strain (520 µg/mL). All applied concentrations of Gal-3 decreased viability of *A. piperis* by 30% (Fig.1), while other black *Aspergilli* were resistant to Gal-3. Additionally, black *Aspergilli* were treated with LPS because it may remain in solution after the production of Gal-3. It caused dose-dependant slight increase in viability of black *Aspergilli* (Fig. 1).

Table 1. The MIC values of amphotericin B and galectin-3 after the treatment of black *Aspergilli*

<i>Aspergillus (Nigri)</i>	MIC AMB (µg/mL)
<i>A. brasiliensis</i> ATCC 16404	1
<i>A. piperis</i>	0.5
<i>A. tubingensis</i>	0.25
<i>A. welwitchiae</i>	0.25
<i>A. niger</i>	0.25
<i>A. luchuensis</i>	0.25

Conclusion. Our preliminary results shows that Gal-3 may have antifungal activity against black *Aspergilli* that is species related and does not involve binding to ergosterol

References

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