Stradam, a novel natural antifungal from the Canadian Arctic microbiome

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An approach to counter the rise in the rate of invasive fungal infections and the emergence of resistance to commonly available antifungals is to mine the biodiversity of underexplored microbial ecosystems for novel agents.[1, 2] Screening of microbial isolates from a recent expedition by our group to the Axel Heiberg Island, Nunavut in the Canadian Arctic identified a novel *Streptomyces* species that exhibited antifungal activity.

MATERIALS AND METHODS

The antifungal activity of a crude organic extract of the novel arctic *Streptomyces A28* was tested against a panel of human fungal pathogens using CLSI methods. The extract's effect on *Aspergillus* was examined by growing *Aspergillus fumigatus* wild type Af293 in RPMI 1640 in the presence or absence of the extract for 21 hours, at 37˚C and 5% CO_2. The samples were stained with calcofluor white and visualized with confocal microscopy. Chromatography coupled with the XTT assay and mass spectrometry was used to purify the antifungal active agent of the extract, termed Stradam. Cytotoxicity of the semi-purified Stradam was assessed against the epithelial cell line A549 with the lactose dehydrogenase assay, and against sheep red blood cells with the haemolysis assay.

RESULTS

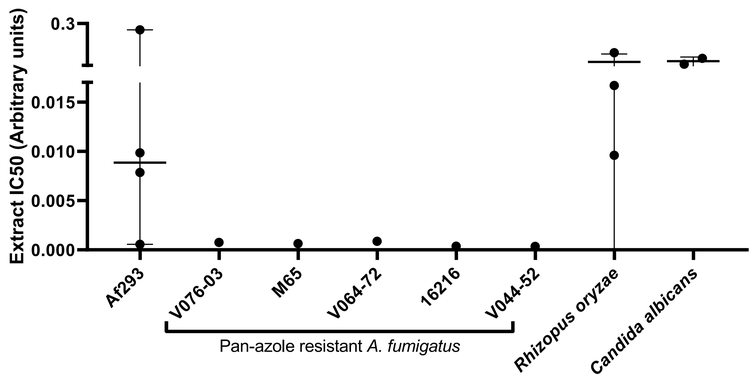

Figure 1: Organic extracts from *S. A28* exhibit a broad range of activity against major human fungal pathogens. The antifungal activity of *S. A28* organic extracts was determined following CLSI methods, with Amphotericin B and posaconazole as antifungal controls. IC50s against a panel of human fungal pathogens were determined. Fungi tested included *A. fumigatus* wild type Af293 and pan-azole resistant clinical strains, *R. oryzae* and *C. albicans*.

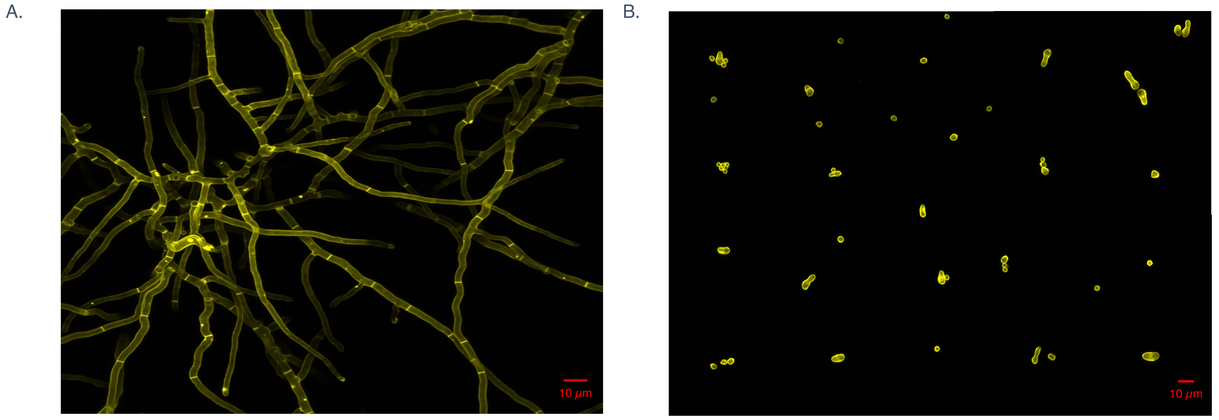

Figure 2: Treatment with *S. A28* organic extracts arrests the germination of *A. fumigatus* conidia. *A. fumigatus* Af293 was grown for 21 hours in the presence (A) or absence (B) of inhibitory concentrations of *S. A28* organic extracts. Hyphae were stained with calcofluor white (yellow) for visualization by confocal microscopy. A: *A. fumigatus* grown in absence of *S. A28* organic extracts exhibited germination and extensive hyphal growth. B: 75% of observed conidia in presence of *S. A28* organic extracts exhibited a marked inhibition of germination and hyphal growth.

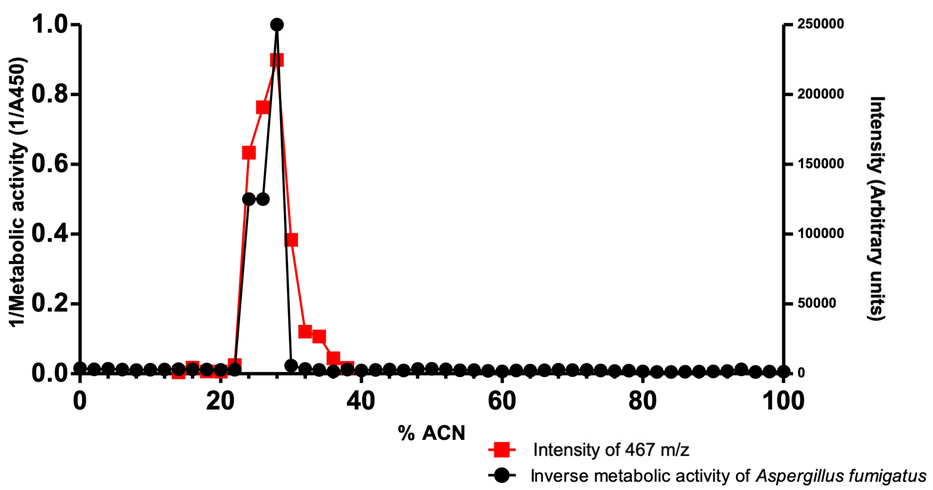

Figure 3: The antifungal activity of *S. A28* organic extract fractions correlates with the presence of a single compound at 467m/z. Elution fractions of the *S. A28* organic extract were tested against Af293 to identify fractions with antifungal activity, shown as 1/metabolic activity. These fractions were analyzed by MALDI-TOF mass spectrometry. Fractions exhibiting antifungal activity, were found to contain a unique peak at 467 m/z. This putative molecule was termed Stradam for future assays.

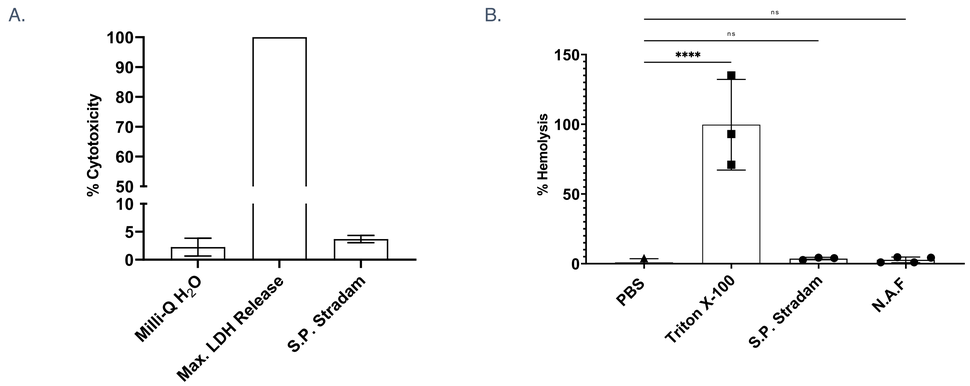

Figure 4: Semi-purified Stradam does not exhibit cytotoxicity against the epithelial A549 cells or red blood cells. A: Semi-purified Stradam (S.P. Stradam) was tested against A549 cells using concentrations of Stradam with inhibitory activity against Af293. Only low levels of cytotoxicity were observed with these preparations. B: Semi-purified Stradam (S.P. Stradam) and the non-antifungal elution fractions (N.A.F) were tested against sheep red blood cells at inhibitory concentrations against Af293. Percent hemolysis for semi-purified Stradam was not significant when compared to the negative control PBS.

CONCLUSIONS

Our studies have identified a potential novel antifungal agent with activity against azole-resistant strains of *A. fumigatus* and *R. oryzae.* Further studies to define the structure of this molecule, and its potential mechanism of action are ongoing.

1. Bongomin F, Gago S, Oladele RO, Denning DW. 2017. Global and Multi-National Prevalence of Fungal Diseases-Estimate Precision. Journal of fungi (Basel, Switzerland) 3:57.
2. Sanchez Armengol E, Harmanci M, Laffleur F. 2021. Current strategies to determine antifungal and antimicrobial activity of natural compounds. Microbiological Research 252:126867.