Evaluation of in vitro activity of *C. neoformans* isolated from hospital samples with synthetic peptides

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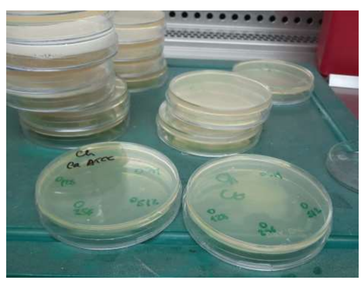
Introduction

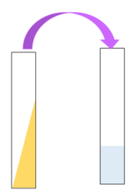
Cryptococcosis is an opportunistic infection caused by *Cryptococcus neoformans* that mainly affects HIV patients. Increased resistance and new strain emergence make necessary new antifungals, like short peptides. Natural antimicrobial peptides (AMPs) are critical components of innate immunity with the function of protecting the host against a wide range of microorganisms, including bacteria, fungi, protozoa, and viruses In this work, three short peptides (P1, P2, and P3), analogs from an amphibian family of AMPs were synthesized by solid phase synthesis (SPPS) and tested as potential antifungal agents against clinical strains of the species *C. neoformans*.

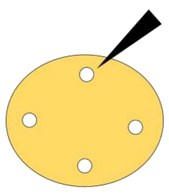
Methods

Peptides were synthesized using Fmoc chemistry on Rink-Amide-methylbenzhydrylamine (MBHA) resin in SPPS. Petri dishes were prepared with Mueller Hinton medium and the inoculum of *C. neoformans* was seeded with each strain to be tested, making holes into which 15 µL of each peptide at different concentrations were inoculated. The plates were incubated for 48 hours at 28°C.



Colonies grown on Saboureaud medium were taken to prepare Mc Farland inoculum.





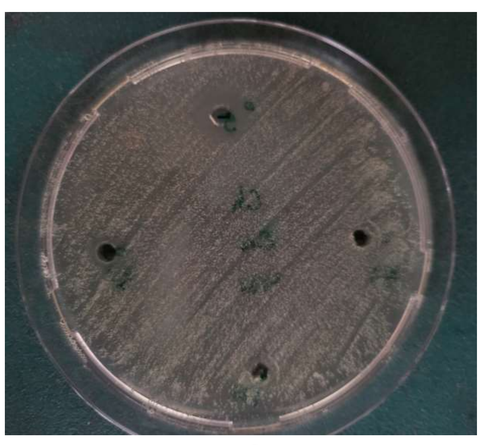
1. Plates were prepared with Mueller Hinton medium, and the inoculum of each strain was seeded.
2. 30 µL of four concentrations of each peptide (5 mg/mL, 2.5 mg/mL, 1.25 mg/mL and 0.512 mg/mL) were inoculated.

Results and Conclusions

Zone of inhibition in the plates with *C. neoformans* produced by the peptides a) P1, b) P2 y c) P3.

P1

P2



P3

- P2 presented more inhibition effect, producing zones of inhibition at a concentration of 1.25 mg/mL.
- P1 y P3 produced inhibition growth at concentrations of 2.5 mg/mL (see table).
- Also, P2 produced inhibition at the lowest concentration analyzed (0.512 mg/mL).

	5 mg/mL	2,5 mg/mL	1,25 mg/mL
P1	15.80 mm	8.57 mm	
P2	21.59 mm	17.80 mm	9.83 mm
P3	12.68 mm	6.51 mm	

Zone of inhibition in mm of *C. neoformans* growth. Peptide concentrations were 5 mg/mL, 2,5 mg/mL y 1,25 mg/mL.

- This is the first time these three peptides were tested as potential antifungal in clinical strains of *C. neoformans*.
- Peptides proved to be an interesting alternative in the search for new antifungals since they present advantages such as less development of resistance compared to commercial antifungals.
- Although the three peptides showed antifungal activity, the minimal concentrations needed for inhibition is higher than commercial antifungals. However, high concentrations would not be relevant for cases of topical application.
- Further experiments should be conducted to reduce peptide concentration, for example, chemical modifications in the sequence, or the addition of lipids or unnatural amino acids that could improve antifungal activity.