MYCOSES **45,** 19–21 (2002) ACCEPTED: MAY 25, 2000

Antifungal activity of *Crotalus durissus cumanensis* venom

Antimyzetische Aktivität von *Crotalus durissus cumanensis*-Gift

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Key words. *Candida albicans*, *Sporothrix schenckii*, *Crotalus durissus cumanensis*, antifungal activity, snake venom.
Schlüsselwörter. *Candida albicans*, *Sporothrix schenckii*, *Crotalus durissus cumanensis*, Antimyzetische Aktivität, Schlangengift.

Summary. The susceptibility to *Crotalus* venom of 14 yeast and 10 mould fungal isolates was assessed. This venom was tested in a standardized well diffusion test, using 400 μg/20 μl well. The percentage of susceptibility to yeast isolates was 78.6% (>8 mm); that for filamentous isolates was 50% (>8 mm).

Zusammenfassung. Die Empfindlichkeit von 14 Hefe- und 10 Hyphomyzeten-Isolaten für *Crotalus*-Schlangengift wurde in einem standardisierten Agardiffusionstest mit 400 μg Gift/20 μl Stanzraum untersucht. Als empfindlich wurden Hemmhöfe >8 mm angesehen. Nach diesem Bewertungsmaßstab erwiesen sich 78.6% der Hefen und 50% der Fadenpilze als empfindlich.

Introduction

The increasing resistance of some fungal infections to antifungal drugs is a major Public Health problem. Advances are being made in recognizing the microbial activity of biological substances derived from reptiles [1]. Among the potential substances for new modes of chemotherapy is snake venom, which appears to mediate processes of microbial degradation. Venom capable of killing or damaging humans is now providing fractions that annihilate bacteria and parasites [2]. The limitations of antifungal chemotherapy underscore the need for new drugs, ideally directed against new targets. This paper discusses available snake venom and summarizes experimental results that support the development of venom as an antifungal substance.

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Materials and methods

Isolates

Yeast and filamentous isolates were obtained from patients at the Mycology Department, Tropical Medicine Institute of the Universidad Central de Venezuela, suffering from different mycoses. Fungi were isolated from throat sputum, venous catheter urine, nails and skin ulcers. All isolates were grown on Sabouraud glucose agar [3] for 24 h at room temperature until adequate growth for the yeast (*Candida* and *Cryptococcus*) or for six or more days for the rest of the hyphal species (*Sporothrix, Penicillium, Aspergillus, Mucor, Rhizopus, Rhinocladiella*).

Yeast from inocula were prepared in sterile 0.85% saline and the suspension turbidity was adjusted to match a 0.5 McFarland barium sulphate standard final concentration [4, 5]. The conidia (hyphal form inocula) from the colony surface were removed by washing the agar slants with sterile 0.85% saline. The conidia solution was then adjusted to a McFarland 0.5 standard turbidity.

Venom

A pool of crude venom from six different specimens of *Crotalus durissus cumanensis* venom was used. The venoms were centrifuged at 2000 *g* to remove cellular debris, lyophilized and stored at −70 °C until use. The stock solution was adjusted to different concentrations: 40, 20,

10 and 0.4 mg ml^{-1} giving concentrations in the 20 µl for each well of 400 µg, 200 µg, 100 µg and 40 µg, respectively.

Well diffusion test [6–8]

The preparation of test plates was as follows: 20 ml Bacto Casitone agar (Difco, Detroit, MI) was melted and cooled to 55 °C to inoculate 1 ml of the fungus suspension. The inoculated agar was poured onto an assay plate (9 cm diameter), and allowed to cool to room temperature. Once the medium was solidified, 4-mm diameter holes were made in the central part of the agar plate and 20 µl of crotalic venom was poured into each hole. Plates were incubated at room temperature for 24 h (yeast) or for more days until adequate growth was present (moulds). After incubation, the diameters of the inhibition-cleared zones were determined.

Results and Discussion

The results of the experiments are summarized in Tables 1 and 2. A total of 24 fungus strains, 10 filamentous fungi and 14 yeasts were studied. With the exception of *Candida dubliniensis* (S-645), *Cryptococcus neoformans*, *Penicillium* spp. (m) and *Fusarium* spp., the growth of 20 isolates (83.3%) were inhibited by the *Crotalus durissus cumanensis* venom.

Since breakpoints were not available to interpret test results for snake venoms, diameters $\geq$8 mm were regarded as susceptible, according to our experience with susceptibility methods [9, 10].

Fig. 1 shows a well diffusion test example of *Crotalus durissus cumanensis* venom against *Candida parapsilosis*. *Sporothrix schenckii* showed marked susceptibility to the same venom (Table 1 and Fig. 2). In the other fungal strains venom susceptibilities from null to scarce were observed (Table 1).

A comparison between the susceptibilities of yeast and filamentous strains to *Crotalus durissus cumanensis* venom is shown in Table 2.

The increase in the use of antifungal therapy, with the emergence of resistance of some pathogenic fungal strains, has made the search for new therapeutic agents an important medical challenge. The antifungal actions of snake venom have not been previously studied. Despite intense

Table 1. *In vitro* sensitivity of different fungal strains to 400 µg/20 µl *Crotalus durissus cumanensis* venom

Species	Diameter (mm)
Candida albicans (90028)	15
Candida albicans (I 46)	11
Candida albicans (I 44B)	12
Candida krusei (6258)	6
Candida parapsilosis (22019)	10
Candida parapsilosis (312)	15
Candida dubliniensis (S2–6)	10
Candida dubliniensis (S 545)	0
Candida tropicalis (156)	10
Candida tropicalis (164)	10
Candida glabrata (6117)	12
Candida glabrata (S639)	10
Candida spp.	10
Cryptococcus neoformans	0
Sporothrix schenckii (21076)	14
Penicillium spp. (v)	6
Penicillium spp. (m)	0
Penicillium spp. (r)	8
Aspergillus terreus	6
Aspergillus spp.	6
Mucor indicus	8
Fusarium spp.	0
Rhizopus spp.	8
Rhinocladiella aquaspersa (322)	8

Table 2. Comparison of sensitivity to *Crotalus durissus cumanensis* venom between yeast and filamentous strains

Species	Total no. strains	Sensibility (mm diameter)			
		0–7	%	8–15	%
Yeast	14	3	21.4	11	79.6
Hyphomycetes	10	5	50.0	5	50.0

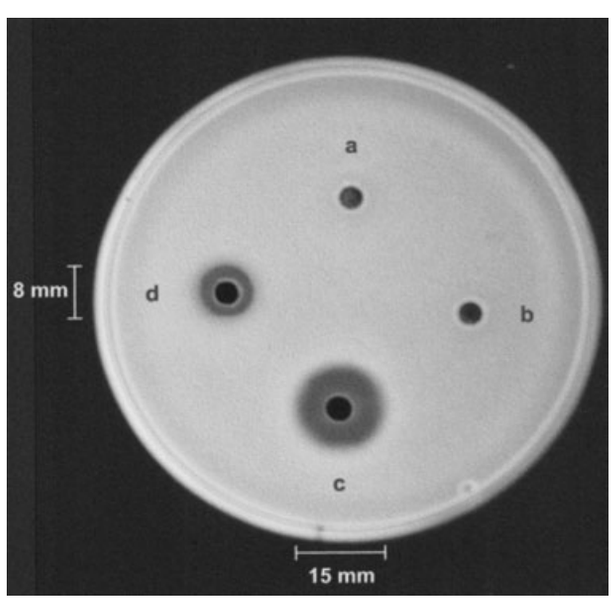

Figure 1. Well diffusion test susceptibility of *Candida parapsilosis* with *Crotalus durissus cumanensis* venom standardization: (a) 40 µg; (b) 100 µg; (c) 400 µg; (d) 200 µg (per well).

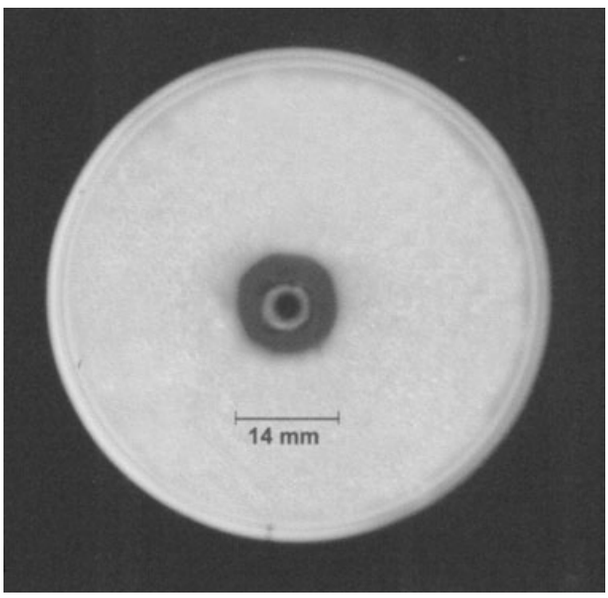

Figure 2. Well diffusion test susceptibility of *Sporothrix schenckii* filamentous phase to *Crotalus durissus cumanensis* venom (400 μg/well).

contamination by potentially pathogenic germs, including fungi, the oral secretions of rattlesnakes rarely result in important infection in their bite victims. Phospholipase A2, found in rattlesnakes, decreases the infectivity in viral and bacterial infections [2]. This activity is probably connected to its ability to damage membranes. Antifungal activity may also be linked to the cytotoxic activity induced by metalloproteases and phospholipases on many different tissues. The cytotoxic action of Crotalidae venom was considered to be smaller than that of Elapidae venom, which disrupted cell membranes within the first hour, leading to cell death [2]. Certainly, Crotalidae venom by its metalloprotease activity must produce the same effect. Agar-based techniques, such as the well diffusion test, offer the possibility to assess fungal susceptibility to snake venom in different species from clinical isolates. The antifungal effect on *Candida albicans* growth of *Carica papaya* latex, which is rich in proteolytic activity, is a comparable phenomenon [11, 12].

It may be concluded that the crude *Crotalus durissus cumanensis* venom was effective in inhibiting the growth of fungi such as *Candida* and *Sporothrix*. Efforts should be made to separate and characterize the fractions responsible for the antifungal activity.

Acknowledgements

The financial support from the Consejo de Desarrollo Científico y Humanístico de la Universidad Central de Venezuela (Grant No.09.34.3855.1999) and Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICIT) (Grant: S1 : 96001933) is acknowledged. We thanks Dr María Safar for reviewing the manuscript.

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