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Phospholipase activity in *Cryptococcus neoformans*

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Abstract

Phospholipases have only been detected in a few fungi and yeasts, in particular in *Candida albicans*. Secreted phospholipases are considered by some researchers to be a potential factor of virulence and pathogenicity in *C. albicans*. Twenty-three *Cryptococcus neoformans* strains were tested in order to observe phospholipase production. Twenty-two of the 23 strains tested were able to produce phospholipases, and the ratio diameter of the colony to total diameter of the colony plus zone of precipitation (Pz) ranged between 0.271 and 0.949. *C. neoformans*, just like *C. albicans*, can be divided on the basis of the Pz into different strains according to their virulence and pathogenicity. There also appeared to be a correlation between the phospholipase production and the size of the capsule in the strains isolated from AIDS patients. For this reason, further studies on *C. neoformans* phospholipase activity would be useful in evaluating the virulence of different strains.

Key words: Cryptococcus neoformans, phospolipase, virulence, AIDS

Introduction

The existence of phospholipases in pathogenic fungi has hardly been reported in literature since 1981 [1]. Among opportunistic yeasts, phospholipases have only been detected in *Candida albicans* and *Saccharomyces cerevisiae* [1, 2–9]. Until now, phospholipase activity has not been reported in *C. neoformans*. After *C. albicans*, this is the major cause of opportunistic and disseminated mycotic infections in immunocompromised and in particular in AIDS patients [10–15].

In *C. albicans*, secreted phospholipases are considered by some authors to be related to the pathogenicity and the virulence of the yeast, since such enzymes can damage the cell membrane of the host by degrading phospholipids and lysophospholipids [1, 3–4, 6–9, 16–17]. For this reason, as in *C. albicans*, it would be interesting to examine phospholipase production in *C. neoformans* strains in future studies to determine a possible correlation between phospholipase activity, virulence and pathogenicity.

Materials and methods

Twenty-three *C. neoformans* strains from the Infectious Disease Institute of Torino University's culture collection, prevalently isolated from AIDS patients, were transferred onto fresh malt agar slants and incubated at 25 °C. After 5 days, the strains were tested for their phospholipase production. The size of the *C. neoformans*' capsule, observed in India ink, was measured through an optical Leitz microscope at 1000× magnification.

Phospholipase production

Determination of phospholipase production was performed according to Polak [17]. The test medium consisted of malt agar containing 1 M sodium chloride, 0.005 M calcium chloride and 2% egg yolk (Bacto egg yolk enrichment 50%, 4 ml in 100 ml agar). Twenty-three strains were tested in Petri dishes filled with 20 ml of agar +0.5 ml of water, and 10 μl of thick suspension of each *C. neoformans* strain was placed in the center of the plate after the agar test had set. Measurement

Table 1. Pz of *Cryptococcus neoformans* strains vs capsule size in μm

n	Strain	Average Pz	Pz class	Average capsule size (μm)	Isolation
1	M27	0.319	++++	2.172	no AIDS
2	AS93SC	0.417	++++	2.987	AIDS
3	M47	0.469	++++	2.576	no AIDS
4	AS124VC	0.474	++++	3.020	AIDS
5	M117	0.517	++++	3.333	AIDS
6	M3	0.560	++++	2.625	AIDS
7	AS125RC	0.596	++++	2.475	AIDS
8	M2	0.607	++++	3.333	AIDS
9	A110	0.620	++++	2.626	AIDS
10	AS134MD	0.626	++++	2.677	AIDS
11	MI80	0.648	++++	2.314	AIDS
12	AS123BL	0.650	++++	2.980	AIDS
13	M43	0.655	++++	2.1720	no AIDS
14	AS547	0.677	++++	2.650	AIDS
15	M48	0.689	++++	2.323	AIDS
16	M26	0.690	++++	2.426	no AIDS
17	AS128DSL	0.712	+++	2.001	AIDS
18	AS138DL	0.804	++	1.987	AIDS
19	AS127MM	0.921	+	3.586	AIDS
20	M104	0.949	+	1.414	AIDS
21	M28	1.000	+	2.323	no AIDS
22	CN1	0.652	++++	2.873	AIDS
23	M72	0.271	++++	4.545	AIDS

Pz between 0.90–1.00 (+); Pz between 0.89–0.80 (++); Pz between 0.79–0.70 (+++); Pz < 0.69 (++++).

and calculation of the zone of phospholipase activity (Pz) was performed according to the method described by Price et al. [7]. Phospholipase activity, after 6 days of incubation at 37 °C, was measured in terms of the ratio of the diameter of the colony plus zone of precipitation [7]. Thus, low Pz means high production of the enzyme, i.e. high virulence, while high Pz means low production of the enzyme, i.e. low virulence. The Pz of three separate samples of each *C. neoformans* strain was measured to obtain the average Pz reported in Table 1. The 23 *C. neoformans* strains, according to the value of their Pz coefficient were grouped in these 4 classes: Pz between 0.9 and 1 (+), very high Pz group; 0.89–0.80 (++) high Pz group; 0.79–0.70 (+++) low Pz group; and Pz minor 0.69 (++++) very low Pz group.

Capsule measurement

Ten cells from each of 10 *C. neoformans* strains, were placed in India ink and randomly observed in 10 different microscopic fields and their capsules measured through a Leitz microscope at 1000×. The average capsule size of each strain is reported in Table 1. A statistical correlation was performed between Pz and capsule size in the 18 *C. neoformans* strains isolated from the AIDS patients.

Results

Phospholipase production and capsule size of the 23 *C. neoformans* strains are reported in Table 1. Twenty-two strains, except the M28 strain, produced different phospholipase. This activity, expressed as Pz, ranged between 0.271 and 0.949 (Table 1, Figure 1).

The majority of the strains (18/23) showed a low Pz (high phospholipase production (++++)). Three *C. neoformans* strains showed low phospholipase activity (high Pz (+)), while only one in the (++) and one in the (+++) class respectively, showed a Pz between 0.804 and 0.712 (Table 1). The Pz average in the AIDS and in the non-AIDS strains was 0.633 and 0.627, respec-

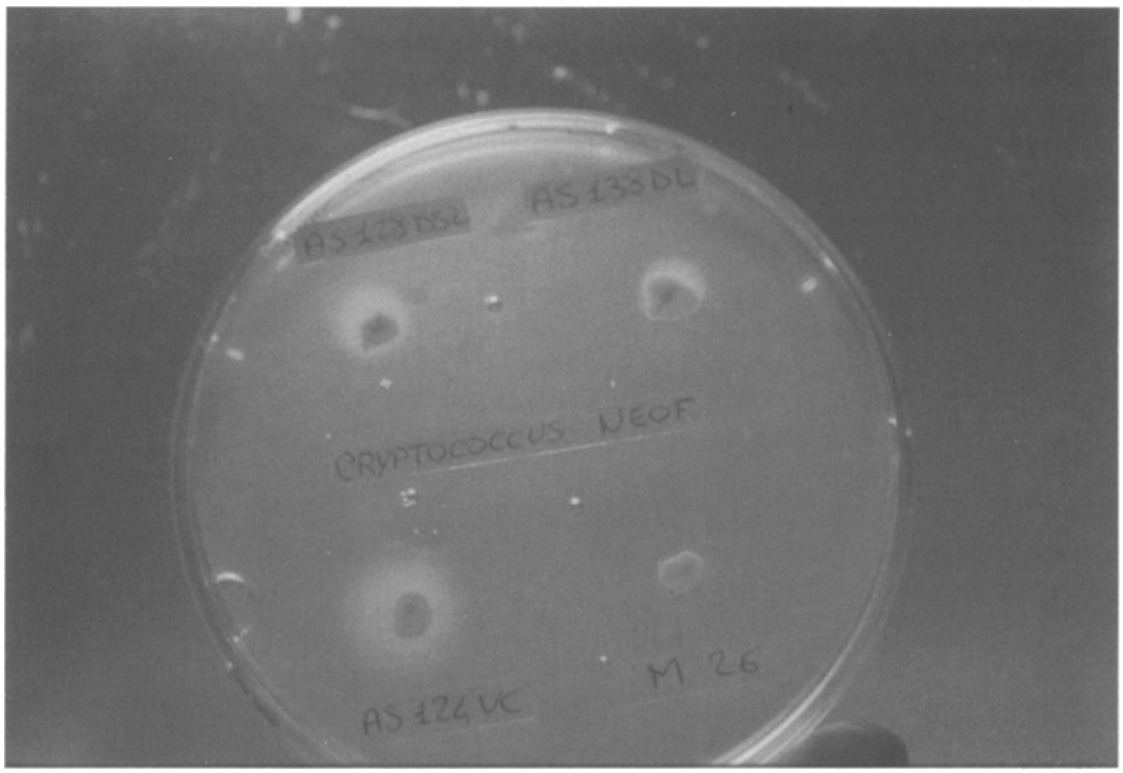

Figure 1. Different phospholipase activities in the *C. neoformans* strains tested.

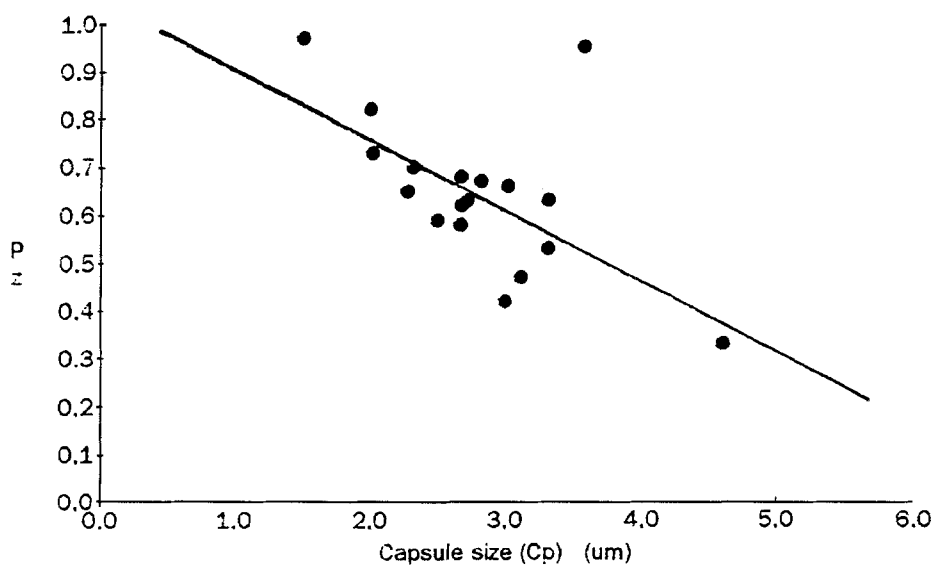

Figure 2. Correlation between Pz and capsule size (Cp) in 18 *Cryptococcus neoformans* strains isolated from AIDS patients. $Pz = -0.147 \times Cp + 1.038$; $r = -0.628$; number of samples = 18; $0.001 < P < 0.01$.

tively. As can be noted in Table 1, the capsule size of the strains isolated from the AIDS patients ranged between 1.414 and 4.545 μm, while that of the non-AIDS strains ranged between 2.172 and 2.873 μm. The average capsule size of the AIDS and that of the non-AIDS strains was 2.76 and 2.33 μm, respectively.

According to the results obtained, the relationship between the Pz and the capsule size (Cp), of the 18 *C. neoformans* strains isolated from the AIDS patients, was expressed as $Pz = -0.147 + Cp + 1.038$, with a correlation coefficient $r = -0.628$ (Figure 2). The r value gave a high correlation probability between the two cellular parameters considered ($0.001 < P < 0.01$). For this reason, it is possible to hypothesize a relationship between phospholipase production and capsule formation in *C. neoformans*, in particular in AIDS patients.

Discussion

It is important to outline that for the first time in the literature, phospholipase activity in *C. neoformans* is being reported. Unlike *C. albicans*, in which phospholipase activity appears after 48 h [6], in *C. neoformans*, it is clearly manifested after 6 days of incubation at 37 °C. This delay in manifesting phospholipase activity in *C. neoformans*, leads one to hypothesize that in this yeast, the phospholipases may be different from those of *C. albicans*.

In the 18 strains isolated from the AIDS patients, the capsule size varied from strain to strain when compared with the non-AIDS strains. According to the results obtained, the capsules of 13 of the 18 strains were thicker than the average capsule size of the non-AIDS strains (2.33 μm).

Moreover, among the AIDS patients, as can be observed in Table 1, 17.8% of the strains showed high phospholipase activity (low Pz) which was correlated to a large capsule, while only 8.7% manifested low phospholipase activity (high Pz) with a small capsule. For this reason, it may also be possible to hypothesize a relationship between the virulence factors of *C. neoformans* and its phospholipase production. Probably, for the generally low Pz observed in the strains isolated from AIDS patients, *C. neoformans* could be more virulent than other strains isolated from non-AIDS patients or from the environment.

Based on their Pz, as with *C. albicans*, *C. neoformans* can also be divided into different strains according to their virulence and pathogenicity. For this reason, further studies on *C. neoformans* extracellular phospholipase activity would be useful in evaluating the virulence of different strains.

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