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The influence of antibiotics on the morphology of Candida albicans and C. stellatoidea

MARIA DYNOWSKA

Department of Botany, Institute of Biology, Pedagogical College, Żołnierska 14, 10-561 Olsztyn, Poland

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The present analyses the influence of antibiotics on the growth modification of *C. albicans* Berkhout and *C. stellatoidea* Langeron et Guerra isolated from human respiratory system.

INTRODUCTION

Preliminary estimation of most cultures in mycological examinations was carried out on the basis of colonies appearance. In the case of yeast fungi of *Candida* type expected distinctive features were not always present especially when antibiotics were added to culture to inhibit the development of bacteria (S c h a b i ń s k i, 1960). This problem was analyzed taking into consideration the observation of *C. albicans* and *C. stellatoidea* strains.

MATERIAL AND METHODS

C. albicans and *C. stellatoidea* strains isolated from respiratory system were examined (D y n o w s k a, 1991-1992). 20 isolates were taken from each species. The culture was carried out on Sabouraud agar with addition of 0.025 % streptomycin and 0.025 % chloromycetin and on Sabouraud agar without antibiotics. According to S c h a b i ń s k i' s recommendations (1960) the first control was carried out after 48 hours of incubation at 37°C and then after a week and 2 weeks of incubation at room temperature. Works of L o d d e r and K r e g e r - v a n R i j (1967) and

R i e t h (1983) were used for strain identification. The colouring of a colony and the structure of its surface as well as the shape and size of vegetative cells were taken into consideration.

RESULTS AND DISCUSSION

Significant influence of antibiotics on the examined fungi morphology was observed in the present investigations. The colonies when streptomycin and chloromycetin were added, did not differ macroscopically after one week. All of them were smooth and shining. Vegetative cells reached larger dimensions (Tab. 1). In the second week of incubation the surface of *C. albicans* creased (Fig. 1a) and resembled the colony of *C. stellatoidea* on the breeding-ground without antibiotics (Fig. 1c, d).

Table 1

The influence of antibiotics on the morphology of fungi

Species	Time of growth (days)	Signs of colony					
		colour		structure of surface		magnitude of vegetale cells (μm)	
		antibiotic					
		+	−	+	−	+	−
Candida albicans Berkhoud	2	white	cream coloured	smooth shinging	smooth shinging	3.5-4.0 x 4.5-6.0	2.0-3.0 x 3.0-4.0
	7	cream-white coloured	"	"	"	4.5-5.5 x 4.5-8.0	3.0-4.0 x 3.5-6.5
	14	"	"	smooth shinging wrinkled	"	"	"
Candida stellatoidea Langeron et Guerra	2	cream coloured	cream coloured	smooth shinging or dull	smooth shinging	3.5-8.0 x 5.0-7.0	3.0-4.0 x 3.5-5.5
	7	"	"	"	craters wrinkled shinging	5.0-7.0 x 8-14 (16)	3.5-6.0 x 5.5-8.0
	14	cream-beige coloured	"	"	"	5.0-7.0 x 8-10 (14)	"

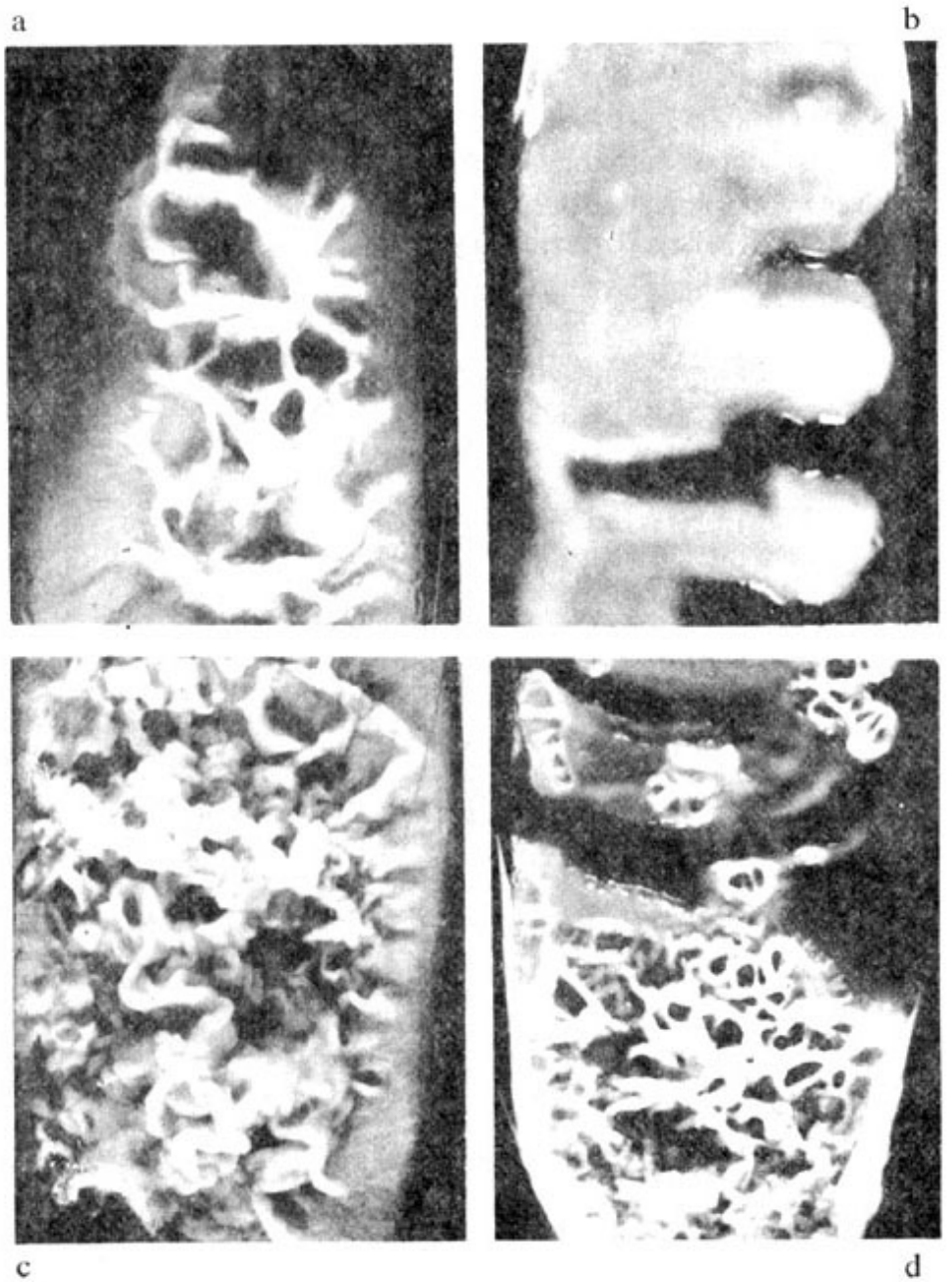

Fig. 1. The growth of analyzed fungi on Sabouraud agar
a – *C. albicans* with antibiotics; b – *C. albicans* without antibiotics; c, d – *C. stellatoidea* without antibiotics

Significant macroscopic differences between species appeared in the colonies grown on pure Sabouraud agar (Fig. 1b, c, d). The following strains of *C. albicans* were observed on the breeding-ground with antibiotics (Fig. 2a, b, c): 1) small and large round and oval cells (probably haplophase), 2) large cells with 1 or 2 buds, 3) large cells surrounded by 3-5 tiny cells, 4) chlamydospores in different stages of development, 5) long 2-nuclei biscuit shaped cells (Fig. 2b) and 6) pseudohyphae with different inner structure (Fig. 2c). M a c i e j o w s k a - P o k a c k a (1976 a) described similar elements in *C. albicans* and *C. tropicalis* (Cast.) Berkhout.

Attention should be paid to the germination of asporogenous (Fig. 2d) and formation of products resembling dangeardien (Van der Walt, Johannsen, 1973; Maciejowska-Pokacka, 1976 b). They were observed on both breeding-grounds. Strains wthout antibiotics formed characteristic pseudomycelium with grape-like blastospores and large round chlamydospores (Kurnatowska, 1978; Rieth, 1983; Dynowska, 1991-1992). On the breeding-ground with antibiotics (Fig. 2c) pseudomycelium grew and developed intensively, resembling morphologically pseudomycelium of *C. stellatoidea* (Dynowska, l. c.).

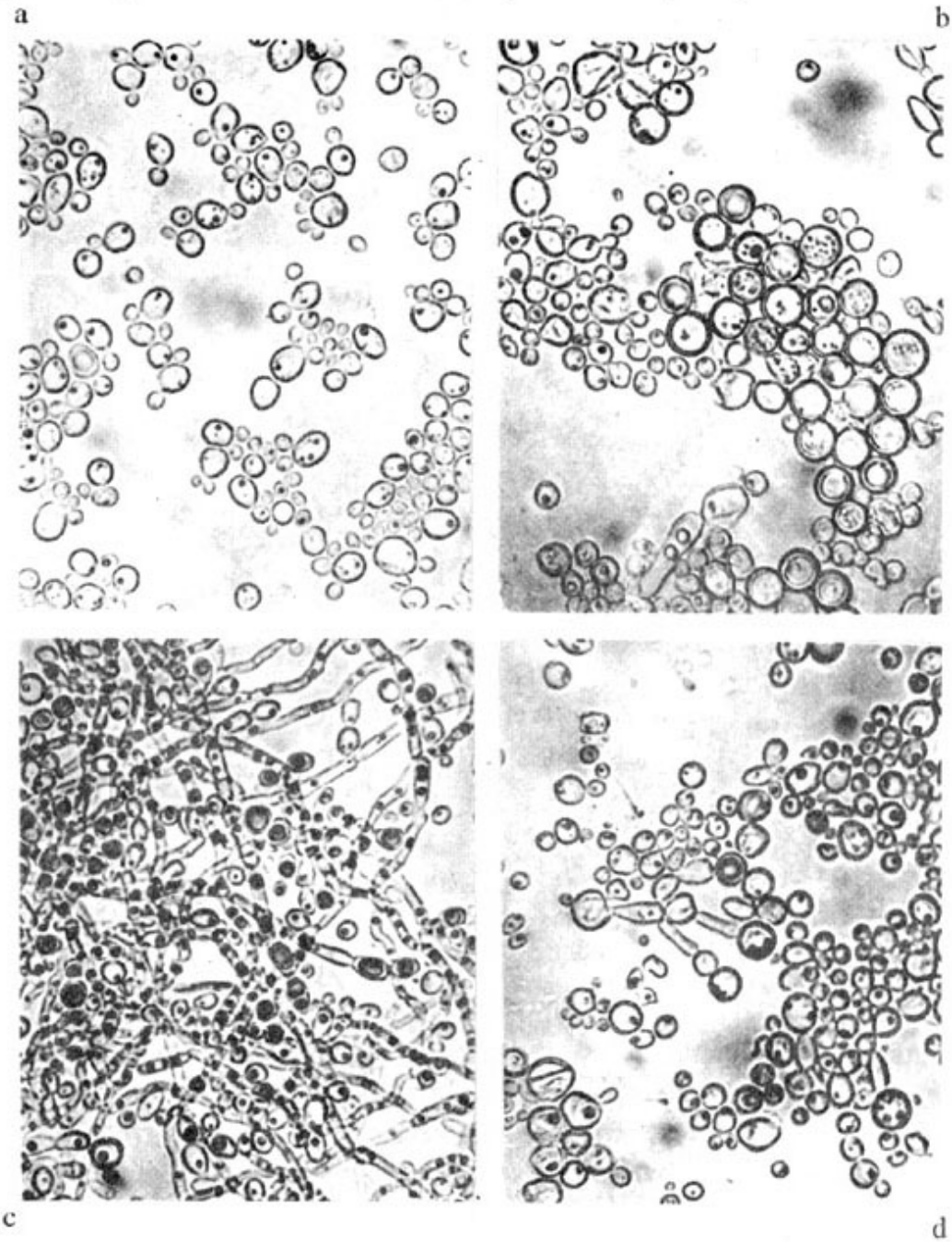

Fig. 2. *C. albicans* – Sabouraud agar with antibiotics
a – cells of various shape, b – chlamydospores and blastospores, c – pseudomycelium, d – a dangeardien

In *C. stellatoidea* strains (Fig. 3a, b, c, d) on the breeding-ground with streptomycin and chloromycetin the following cells were formed: 1) small and large oval and round cells, 2) elipsoidal and biscuit-shaped cells 2 or 3 parts, 3) stalagmoidal cells. Pseudomycelium developed intensively (Fig. 3b, c) forming numerous chlamydospores and endospores (Fig. 3b). The latter were also present in *C. albicans* (Fig. 2c). Lodder and Kreger-van Rij (1967) and Maciejowska-Pokacka (1976 a) found them also in other yeast fungi. Lipid bodies appeared in cells (Fig. 3b, d). *C. stellatoidea* developed slower on the agar without antibiotics and formed soft pseudomycelium (Fig. 3f); vegetative cells showed little differentiation (Fig. 3e).

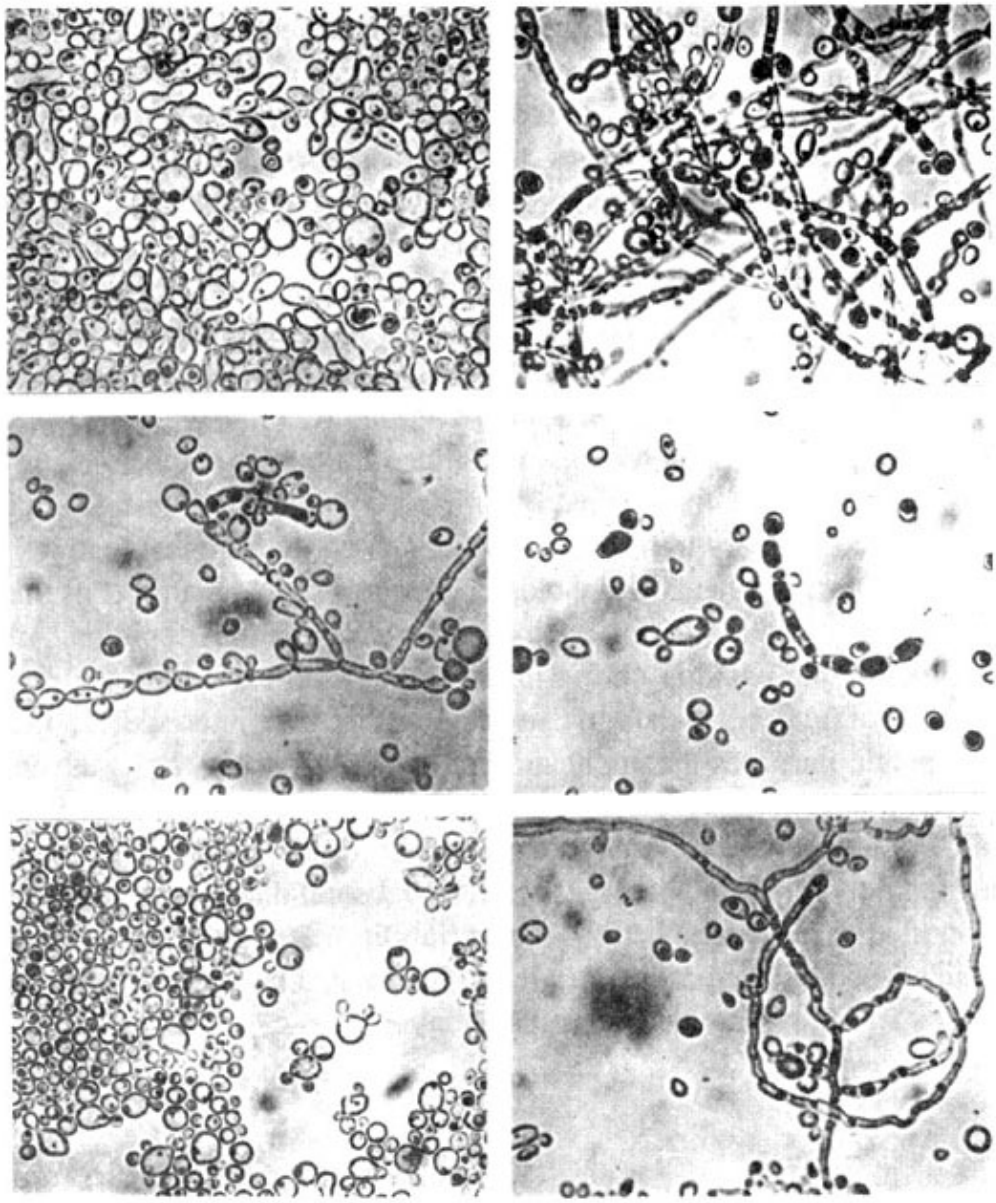

Fig. 3. *C. stellatoidea*

Sabouraud agar with antibiotics: a – cells of various shape, b – pseudohyphae and endospores, c – vegetative cells, d – cells with lipid droplets

Sabouraud agar without antibiotics: e – vegetative cells, f – fragment of pseudomycelium

It should be emphasized that all strains had small and large cells. The small ones were formed as a result of reduction division, the large ones – just as chlamydospores, endospores and pseudomycelium are regarded as a diploidal phase. W i c k e r h a m (1964) investigated perfect stages of *Candida* sp. and differentiated several types of unisexual and bisexual cells. Colonies composed of these cells formed elevations in shape of craters or sectors on the breeding-ground surface. Similar growth was observed in our investigations (Fig. 1a, c, d).

There is a supposition that *C. stellatoidea* is a local variety of *C. albicans* (R i e t h, 1983). Our observations do not seem to prove such a hypothesis. Although these species often appear together in human respiratory system but they differ morphologically (D y n o w s k a, 1991-1992). The differences are particularly clear when we compare them on Nickerson's rendom breeding-ground used in mycological diagnostics (K o w s z y k - G i n d i f e r, S o b i c z e w s k i, 1986). Earlier observations showed that both species can grow close to one another but *C. stellatoidea* dominates over *C. albicans* showing greater vitality (D y n o w s k a, 1991-1992).

The results of these investigations strictly correspond with S c h a b i ń s k i's observations (1960). The author states that antibiotics added to a breeding-ground in order to inhibit the development of bacteria have a significant influence on the growth modification. *C. albicans* looks like *C. crusei* under the influence of penicilin. M a c i e j e w s k a - P o k a c k a (1976 a) who was looking for a relationships between *C. albicans* and *C. tropicalis*, also added antibiotics to the breeding-ground. It is not unlikely that morphological features of colonies were changed due to this. Therefore, it is necessary to take into consideration physiological features of yeast fungi while determing them. Morphological features should not be of primary criterion. M a c i e j o w s k a - P o k a c k a (1976 a) is of similar opinion. Antibiotics destroy undesirable bacteria during investigations and excelerate the growth and development of fungi but at the some time they efface their inter-species differences. For therapeutic purposes the application of antibiotics seems to be adventageous. The problem is to identify them very quickly and to obtain pure, strains without bacteria. For taxonomic purposes pure cultures of the above mentioned fungi should be obtained rather by serial passages on fresh Sabouraud breeding-ground.

Obtained results demonstrate the variability of yeast fungi and continous difficulties faced by diagnostics. At present, investigations on enzymatic activity of *C. albicans* and *C. stellatoidea*, are still continued.

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