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The influence of senescence on some fungi of the genus Candida

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The present paper deals with morphological and physiological changes of some fungi of the genus *Candida* caused by the process of senescence of the culture.

INTRODUCTION

Investigations concerning the possibility of keeping breeding and durability of fungi species features of *Candida* type (Dynowska, 1990; 1991) suggest that after the period of 3 months physiological and morphological features undergo significant changes. They are, therefore the subject of this paper.

MATERIAL AND METHODS

Candida albicans Berkhout, *C. parapsilosis* Langeron et Talice, *C. guilliermondii* Langeron et Guerra and *C. stellatoidea* Langeron et Guerra strains isolated from human respiratory system were examinated. The way of breeding was presented in earlier paper (Dynowska, 1990; 1991). Some strains which were to be used for physiological investigations were incubated on Sabouraud's agar with 0,025 % streptomycin and 0,025 % chloromycetin.

Observation were made after 48 hours and then every week over the period of 3 months. The papers of: Lodder, Kreger-van Rij (1967), Kurnatowska (1978), Rieth (1983) and Kowszyk-Gindifer, Sobiczewski (1986) were used for identifying of species.

RESULTS AND DISCUSSION

The analysis of macroscopic features of 3-month old culture on Sabouraud's agar (Tab. 1) shows that in all cases the colour of colonies gets darker both on the surface and underside in time. The surface becomes mat and creased. The edges are irregular. The shape of the colony is the most stable feature and generally does not change from the period of the second week of incubation. After 2 weeks of breeding cloddy rises appear on the surface of *C. guilliermondii* whereas *C. stellatoidea* creates characteristic craters. According to W i c k e r h a m (1964) it is the result of a sexual process where small and large cells differentiate and then the mycelium developes intensively and unevenly. In time all examined strains except for *C. guilliermondii*, enter into a breeding-ground creating bush like pincers such as *C. parapsilosis* and *C. stellatoides* and tabular like pincers in the case of *C. albicans*. Polish diagnostic literature (K u r n a t o w s k a, 1978; 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) indicates that none of the examined species creaters of colonies, enter into a breeding-ground. This is a feature that should be verified. On the basis of data analysis it can be deduced that macroscopic features stabilize in the second week of incubation.

Similar conclusions can be drawn on the basis of microscopic observations. After 48 hours of breeding vegetative cells and blastospores become small. Only in *C. albicans* and *C. stellatoidea* they become larger. Few chlamydospores have already well developed. Pseudohyphae of *C. stellatoidea* begin to branch. *C. albicans* and *C. guilliermondii* create blastospores in characteristic heads (M a c i e j o w s k a - - P o k a c k a, 1976; D y n o w s k a, 1990; 1991). After 48 hours it is often difficult to differentiate these two species. Incubation must last longer in order to state whether pseudohyphae branch like a tree (*C. guilliermondii*) or not (*C. albicans*). Such a type of growth is rather well observed after 7 days of breeding when pseudomycelium develops intensively. The chlamydospores and characteristic blastospores are much larger: rounded in *C. albicans* and *C. guilliermondii*, egg-shaped and rounded in *C. parapsilosis* and egg-shaped in *C. stellatoidea* (D y n o w s k a, 1990; 1991). Pseudohyphae become thicker. Such a picture is maintained till the 14th day of breeding. Then the most characteristic features of examined species can be observed. Further prolongation of breeding (21st-30th days) leads to the increase in the number of chlamydospores and blastospores and to the decrease in the number of vegetative cells (Tab. 2). Then the chlamydospores of *C. stellatoidea* are similar in appearance and size to blastospores of *C. albicans* after 48 hours of incubation. After 2-3 months the pseudomycelium of all examined species becomes softer and pseudohyphae are tiny. *C. stellatoidea* does not form any chlamydospores. The optimal time for noticing microscopic features seems to be between the 7th and 14th day of incubation.

Physiological analysis of the selected strains (Tab. 2) also shows that as breeding gets older and the enzymatic activity decreased after 21 days. When antibiotics are added to the substratum the initial physiological properties are maintained until

the 2nd month of incubation. In the first 7 days of breeding a partial decomposition of arbutin takes place in all *C. albicans* strains. In some strains lactose is also decomposed (No. 2, 3, 7). In literature (Maciejowska-Pokacka, 1976; Kurnatowska, 1978; De Louvois, Mulhall, Hurley, 1979) the reaction with arbutin is defined as adverse to fermentation and partial decomposition of lactose. Maciejowska-Pokacka (1976) observed a partial decomposition arbutin in *C. tropicalis* (Cast.) Berkhout cosely related to *C. albicans*. The examined *C. albicans* isolates assimilate sucrose very well. In the present investigations on the 14th day of incubation this sugar was only partly fermented and assimilated, and after 21 days was not decomposed. Similar divergences with literature data were noticed in the case of *C. stellatoidea*. According to Lodder, Kreger-van Rij (1970) this species does not ferment lactose and saccharose. In the present investigations the reaction was partly positive in some strains (No. 14, 15, 20). Perhaps it is caused by the formation of examined isolates or creation of new strains with wider possibilities of utilization of compounds from the substratum. When the physiological properties of the studied fungi are taken into account, a similarity between *C. albicans* and *C. stellatoidea* is observed.

The obtained results prove that fermentation and assimilation processes become less intensive when breeding gets older. Roeb (1974) noted that the enzymatic activity of phytopatogenic fungi depended on the age the mycelium. The younger mycelium had more enzymatic activity than the older one.

Strains brought on fresh Sabouraud's agar in order to rejuvenate the breeding, regained their initial features and properties. Enzymatic activity was prolonged by adding antibiotics to substratum, which not only destroyed bacteria but also positively influenced the vitality of the examined fungi.

There is also a question whether fungi, which inhabit the human body longer lose their vitality. If there is a biological balance then the answer should be positive. The explanation of this problem should be sought in immunology. It is difficult to relate the obtained results in vitro to the those in vivo. In the first case compounds of substratum are used up after some time, in the second case — organism gives compounds to fungi all the time. This is an additional problem for chemistry therapeutists. Many drugs giving very good results in laboratory experiments appear ineffective in a living organism (Kowszyk-Gindifer, Sobiczewski, 1986).

It should be pointed out that the identification of the examined fungi ought to be carried out between the 7th and the 14th day of incubation. At this time the morphological and physiological features seem to be stable. In the 3th week the features get loose and after a month there are significant changes. Diagnostic literature (Dermoumi, 1979; De Louvois, Mulhall, Hurley, 1979) advices the estimation of physiological features of yeast fungi after 72 hours of incubation at 30°C on account of development of the studied fungi and the quicker process of their senescence.

Table 1

Macroscopics features of 90-day growings of *Candida* sp. on Sabouraud agar

Species (No of strain)	Time of growing (days)	Shape	Colour		Structure of surface	Other signes
			surface	bottom		
Candida albicans (1-10)	2	round, flat	white	cream	smooth, shining, soft, regular margin	strike not in agar
	7	round, regular, convex	cream	"	"	"
	14	regular, convex	"	beige	smooth, shining, clammy, regular margin	"
	21	"	cream, yelow	coffee	slightly in center pleated, slightly shining, clammy, irregular margin	slightly strike in agar
	30	"	beige	"	clear in center place, mat	"
	60	irregular convex	"	clearly brown	"	cave in top
	90	"	"	"	"	strike in agar wrinkle from the bottom
Candida guilliermondii (31-40)	2	irregular flat	white	white	smooth, mat, soft, clammy	strike not in agar
	7	conical	"	cream	"	"
	14	"	"	"	cloddy, mat	"
	21	irregular conical	cream	beige	slightly wrinkled	"
	30	"	"	"	reticular wrinkled	"
	60	"	cream, beige	"	"	"
	90	"	"	"	"	"

Candida parapsilosis (21-30)	2	irregular flat	clearly cream	darkly cream	smooth, shining, very soft, smooth margin	strike not in agar
	7	flat	cream	"	"	"
	14	"	"	"	regular wrinkled, mat	strike in agar
	21	"	"	yelowish	"	"
	30	"	beige	coffee	intensive wrinkled	"
	60	"	"	"	"	"
	90	"	"	"	"	"
Candida stellatoidea (11-20)	2	round, oval	cream	cream	smooth, shining, mat margin	strike not in agar
	7	conical	clearly beige	darkly beige	in center craterly and wrinkled, soft, smooth margin	"
	14	"	"	"	slightly furrow, regular wrinkled	slightly strike in agar
	21	"	beige	"	hard, inside soft, intensive wrinkled	"
	30	"	"	coffee	"	strike in agar
	60	"	"	"	"	"
	90	"	"	"	"	"

T a b l e 2

Macroscopics features and physiological characteristic of 90-day growings of some selected species of the *Candida* genus

Species (No of strain)	Time of growing (days)	Vegetale cells		Blastospores		Chlamydospores		Pseudohyphae	Fermentation					Assimilation							Splitting of arbutin
		shape	magnitude (µm)	shape	lenght (µm)	shape	diameter (µm)	width (µm)	galactose	glucose	lactose	maltose	saccharose	galactose	glucose	lactose	maltose	saccharose	$(NH_4)_2SO_4$	asparagine	
Candida albicans (1-10)	2	r	2-3 x 3-4	ov	2-6	r	6-8	2-4	+	+	±	+	+	+	+	±	+	+	+	+	±
	7	r, ov	3,5-4,5 x 4,5-5,5	ob, ov	3-8	"	"	3-5 (6)	+	+	±	+	+	+	+	±	+	+	+	+	±
	14	"	"	"	"	"	6-8 (10)	5-7	+	+	+	+	+	+	+	±	+	±	+	+	–
	21	ov	"	"	"	"	"	"	+	+	–	±	–	+	+	–	±	–	+	±	–
	30	ob, ov	2-4 x 2,5-4,5	r, ov	3-4	"	5-6 (3-4)	3-4	±	+	–	±	–	–	+	–	±	–	±	±	–
	60	"	2-3 x 2-4	ov	"	"	4-5	2-3	±	+	–	±	–	–	+	–	±	–	±	–	–
	90	"	"	"	2-3	"	"	"	–	+	–	±	–	–	+	–	±	–	±	–	–
Candida guilliermondii (31-40)	2	c	2-5 x 3,5-6	"	"	–	–	2,5-3	+	+	–	+	+	+	+	–	+	+	+	+	+
	7	od	2,5-5 x 3,5-7,5	r, ov	2-4	–	–	2,5-4,5	+	+	–	+	+	+	+	–	+	+	+	+	+
	14	"	3-5 x 4-8	ov	3-5	–	–	2,5-5	+	+	–	+	+	+	+	–	+	+	+	+	+
	21	"	"	"	"	–	–	"	+	+	–	+	+	+	+	–	+	+	+	+	+
	30	c, ov	3-5 x 5-6	"	2-3	–	–	2-4	±	+	–	+	±	±	+	–	+	±	±	+	+
	60	ov	2-5 x 3-5	"	"	–	–	2-2,5	–	+	–	+	–	–	+	–	±	–	±	±	±
	90	"	2-4 x 3-4	"	"	–	–	"	–	±	–	+	–	–	±	–	±	–	±	–	–

Candida parapsilosis (21-30)	2	od, c	2,5-5 x 4-8	r, ov, od	1,5-2	r	–	–	+	+	–	+	±	+	+	–	+	+	+	+	–
	7	od, c, r	3,5-4 x 6,5-12	"	2-2,5	–	–	1,5-3	+	+	–	+	±	+	+	–	+	+	+	+	–
	14	"	3-5 x 4-8	"	3-6	–	–	2-4	+	+	–	+	±	+	+	–	+	±	+	+	–
	21	"	3-5 x 4-10	"	"	–	–	"	±	+	–	+	–	+	+	–	+	±	+	+	–
	30	od, c	2-4 x4-7	ov	3-4	–	–	2-3	±	+	–	+	–	+	+	–	+	–	+	+	–
	60	r	2-3 x 3-4	"	2,5-3	–	–	"	–	+	–	±	–	±	±	–	±	–	+	±	–
	90	"	"	"	2-3	–	–	1,5-2	–	±	–	±	–	–	±	–	±	–	±	–	–
Candida stellatoidea (11-20)	2	od	3-4 x 5-7	od	3-5	od	5-6	4,5-6	+	+	±	+	+	+	+	±	+	+	+	+	+
	7	od, c	4-8 x 5-12	od, ob	3-6	"	4-6,5	4,5-8	+	+	±	+	+	+	+	±	+	+	+	+	+
	14	"	5-7 x 8-14	od	3,5-6	"	5-8	"	+	+	±	+	+	+	+	±	+	+	+	±	+
	21	od	5-6 x 4-7	"	4,5-5	"	4,5-7	5-7	±	+	±	+	±	–	+	–	+	+	+	±	±
	30	r, od	3,5-6 x 3,5-6	ov	3,5-4	od, r	4-6	4-6	±	+	–	+	±	–	+	–	+	–	+	–	±
	60	"	3-5 x 3,5-5	"	3-3,5	r	4-5	4-4,5	–	+	–	+	–	–	+	–	+	–	±	–	–
	90	r	2,5-3 x 3-3,5	"	"	–	–	3,5-4	–	+	–	±	–	–	+	–	±	–	±	–	–

c – cylindric, ob – oblong, od – ovoid, ov – oval, p – round

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