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INVESTIGATION OF BIOLOGICAL PROPERTY FEATURES VARIABILITY OF CANDIDA FAMILY YEAST-LIKE FUNGI

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Abstract
The purpose of investigation was to study cultural and proteolytic properties of candida family yeast-like fungi. It is stated, that natural variability of C.albicans colonies morphology depends on storage period of strains and amount of passages. Besides typical S and atypical K forms, R forms can appear both at collectional, and at hospital strains. Negative results on proteolytic activity are determined for collectional strains in the third passage, for hospital strains in the first passage.

Keywords. Microbiology, genus candida, typical S, atypical K, collectional, morphology.

INTRODUCTION

Experts in the field of microbiology and infectious diseases consider yeast fungi of the genus Candida as conditionally pathogenic microorganisms and believe that they are found in the human body in a normal state and are activated as a result of various exogenous influences and changes (Dexkankhodzhaeva N.A., 1995; Yelinov N.P., Vasilyeva N.V., 2000; Mukhamedov I.M., 2004; Bailey A. et al., 1995; Chaffin W.L., 1999).

In the classification of microorganisms, the genus Candida is a separate genus, about 80 species are known (Koch H., 1973), only a few of which can cause disease in humans (Tonoto K., et al, 2004). These include C. albicans, C. tropicalis, C. pseudotropicalis, C. krusei, C. guillermondi, C. pelliculosa, C. parapsilosis.

Yeast fungi of the genus Candida are conditionally pathogenic microorganisms that can be found in the skin and mucous membrane of the human body (Ishakova X.I. and 1986; Bazhenov L.G., 2002, 2005). These microscopic fungi are found in 30-50% of healthy people's sputum, urine, feces, and when cultured from the mucous membrane of the skin. In the researches of Rebrova R.N., (1989) it was determined that its occurrence in the mucous membrane of the human oral

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cavity increased to 46-52%. In non-pregnant women, the incidence of vaginal mucosa reaches 11-13%, but it increases sharply in the third pregnancy, ranging from 29% to 86% according to various data. The frequency of meeting Candida fungi in feces is 80%, and up to 9% in uninjured skin. Candida carriage in clinically healthy people is up to 5%, in 53.2% of those with colds of the mucous membrane (Rebrova RN, 1989).

Methods and Materials

Cultivation and quantification of yeast fungi of the genus Candida is important in diagnosing candidiasis infections and determining the level of dysbiosis in different biotopes of the body. A change in the environmental conditions of yeast fungi of the Candida genus causes a change in their various biological properties. In particular, it changes the cultural, proteolytic, adhesiveness and other properties of fungi. This reduces their production in terms of quality and quantity.

With this in mind, the purpose of this study is studying the cultural and proteolytic properties of yeast fungi of the genus Candida under the influence of various factors. Materials and methods. We studied the morphological, cultural and proteolytic properties of Candida albicans from the main species of Candida genus. Identification and differentiation of the isolated microorganism was carried out according to Bergy (1997). Collection and hospital strains of Calbicans were performed 3 times to compare results.

The grown colonies of standard strains of the microorganism (10^{-6} - 1000 microbial cells in 1 ml, 10^{-7} - 100 microcells in 12 ml) were quantified.

Research Results

We proposed a method to simplify the assessment of the growth of yeasts of the genus Candida grown on rice sawdust-based media. We proposed to carry out a qualitative assessment of the growth characteristics of C.albicans colonies grown in a nutrient medium on the basis of a special scale (Nuraliev N.A. 2006).

Level I - good growth (colonies are typical, full, mature and well visible, abundant growth when incubated at 37^0C for 18-24 hours, met the requirements of nomenclature. Morphological, tinctorial, enzymatic of microorganism cultures and other biological properties have not changed);

Level II - satisfactory growth (colonies tend to be small, 4-6 hours behind the development to typical appearance at 37^0C for 18-24 hours, but morphological, tinctorial, enzymatic and other biological characteristics of cultures of microorganisms unchanged);

Level III - weak growth (colonies are very small, visually difficult to identify. Does not meet the standards of nomenclature, it is difficult to monitor growth under the conditions of detection by traditional methods);

Grade IV – no visible growth (Table 1).

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Table 1 Evaluation of the growth characteristics of standard cultures of Candida yeasts grown in SHQSE-1 and SHQSE-2 on a special scale

Culture, list number	SHQSE - 1				SHQSE – 2			
	After 24 hours		After 48 hours		After 24 hours		After 48 hours	
	Consentration							
	10^4	10^2	10^4	10^2	10^4	10^2	10^4	10^2
Candida albicans 7 003838	II	IV	I	II	II	IV	I	I
Candida albicans 10 003848	II	IV	I	II	II	IV	I	I
Candida albicans 5 003818	I	II	I	I	I	II	I	I
Candida albicans 723 003592	I	II	I	I	I	II	I	I

SHQSE was prepared in 2 variants: in nutrient juice (SHQSE-1) and in isotonic solution of 0.5% NaCl (SHQSE-2).

In addition, we studied the natural variability of the colony morphology of standard and hospital strains of Candida albicans SHQSE-1 and SHQSE-2. The following colony types were found in the Candida albicans population in the first passage. Its morphology is typical (S appearance) and atypical - deafness (K appearance). As the number of passages increases, the number of S-form collection strains increases, while the number of K-form colonies decreases. This shows that the colonies of the deaf form are not stable and have a phenotypic character of variability (Table 2).

Table 2 Natural variability of colony morphology in Candida albicans populations

Passage	Morphological appearance of colonies					
	Collectionstrains			Hospital strains		
	S	K	R	S	K	R
I	76±2.8	24±1	0	95.2±1.4	4.8±1.2	0
II	82±2.4	18±1.3	0	96.7±1.5	3.3±1.4	0
III	82±1.8	17.2±1.8	0.8±0.6	88.4±2	11.2±1.6	0.4±0.4

Typical colonies are smooth, raised, shiny, with smooth edges, white in color; On the third day at 370C, in Saburo medium with 4% glucose, the diameter size is 3-7 mm. In atypical colonies, all symptoms are similar, and on the third day, the diameter of the colony is up to 2 mm.

We observed the opposite when nosocomial strains were studied. As the number of passages increased, the percentage of typical colonies (S appearance) of hospital strains decreased ($r<0.05$), K appearance increased. The frequency of these manifestations increased by 2.3 times in hospital strains in III-passage compared with I-passage ($r<0.001$).

In all cases, the appearance of K was the same. Our findings show that, firstly, Candida albicans hospital and reference strains grew optimally in the proposed environment; second, collection strains are more resistant to repeated passages than hospital strains.

In addition, in passage III, colonies of a different morphological appearance appeared (R appearance). These colonies are radially linear, the surface is uneven, they protrude from the

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surface of the nutrient medium, they are white in color, up to 4 mm in diameter, the frequency of occurrence is 0.4-0.8%.

The next stage of our work was a comparative study of the change of proteolytic activity in the collection and hospital strains of Candida albicans (Table 3).

In the collection strains, a higher percentage of proteolytic activity in the I-passage was observed in colonies of the K form compared to the S form ($p<0.05$). In passage II, the same cultural situation was preserved for the appearance of K. It should be noted that in II- and III-passages, the opposite result was observed in S-type collection strains - 4±3.3% (in the absence of high and medium proteolytic activity, low proteolytic activity in 96±8%). No such change was observed in K appearance.

Table 3 Variability of proteolytic activity of Candida albicans collection strains of different forms

Passage	Proteolytic activity level					
	High		Average		Low	
	S	K	S	K	S	K
I	10±3.2	19.3±3	18±4	42.2±6.3	72±5.2	38.5±4
II	0	6.5±2.8	30.4±4	39.4±5	69.6±5.3	54.1±3.8
III	0	0	0	50±8.3	96±8	50±8

It was found that the percentage of high proteolytic activity in the first passage was 19.3±3% and 10±3.2% higher in K atypical form than in S typical form in the collection strains. The same situation was observed in the second passage of these cultures. In the third passage, a negative result was observed for the proteolytic activity in the S form of the collection strains, and no such specific change was observed in the K form.

4 – table Variability of proteolytic activity of different forms of Candida albicans hospital strains

Passage	The level of proteolytic activity					
	high		average		low	
	S	K	S	K	S	K
I	39.2±7.2	21.1±6	22.1±6	18±5.3	28.6±6.7	32±6.6
II	0	0	40±7.3	30±6.7	60±7.7	70±5.8
III	0	7.3±3.2	74.2±6	60.4±8.2	25.8±4.8	32.3±6.2

In further studies, we observed a different picture when studying the proteolytic activity of hospital strains. Proteolytic activity in the typical form of S was 1.9 times higher than in the atypical form of K. A negative result of proteolytic activity in hospital strains was found in 10.1±4.5% cases of S form and 28.9±4.7% cases of K form. As the number of passages increased, the proteolytic activity always decreased in the S-type colonies, and high activity was not observed in the III-passage.

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Summary

1. Aqueous rice bran extract showed good and moderate growth after 48 hours at concentrations of 10-6 and 10-7 of Candida genus on the proposed scale.
2. Natural variation in the morphology of C. albicans colonies depends on the duration of storage of the strains and the number of passages. In addition to the third passage atypical S appearance and atypical K appearance, R appearance was shown in both collection and hospital strains.
3. Negative results for proteolytic activity were observed as follows: in the third passage of the collection strains, S appeared in 4% of cases, in the first passage of hospital strains, S appeared in 10.1% of cases, for K appearance in 28.9% of cases.

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