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Investigation of Biological Property Features Variability of Candida Family Yeast-Like Fungi

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ABSTRACT

Objective: *Yeast-like fungi of the Candida genus are opportunistic pathogens implicated in candidiasis, yet biological variability – particularly in colony morphology and proteolytic properties – remains underexplored beyond Candida albicans, especially across strain origins, culture passages, and storage conditions.* ***Method:*** *This study investigated the morphological and proteolytic characteristics of Candida spp. from both hospital and collection strains, assessing variability under controlled passage conditions. Evaluations were conducted using a rice bran-based medium and a standardized scale for growth assessment.* ***Results:*** *The study identified distinct colony morphotypes – typical S, atypical K, and rare R forms – whose distribution varied with passage number and storage duration. Notably, collection strains exhibited a significant decline in proteolytic activity by the third passage, while hospital strains showed reduced enzymatic activity from the initial passage.* ***Novelty:*** *This research provides new insights into the phenotypic plasticity of Candida strains, emphasizing the diagnostic relevance of passage tracking. It also introduces a practical and accessible method for evaluating growth characteristics using rice bran-based media, contributing to improved diagnostic accuracy, treatment strategies, and epidemiological monitoring in clinical mycology.*

INTRODUCTION

Candida - yeast-like fungus of the genus Cryptococcoceae, responsible for causing candidiasis. These fungi constitute the usual microflora of several biotopes within the human body, residing there under typical conditions, and are activated due to various foreign effects and alterations. If candidiasis is not diagnosed and treated correctly during the disease, it can lead to serious medical problems[1].

Candida are yeast-like fungi of the Cryptococcoceae genus that exist as part of the normal human microflora but can become pathogenic under certain conditions, causing candidiasis. These opportunistic pathogens inhabit various biotopes of the human body and are activated by external influences or internal imbalances. Candidiasis, if undiagnosed or improperly treated, may lead to severe medical complications affecting multiple organ systems. In microbiological classifications, the Candida genus includes around 80 species, though only a few, such as C. albicans, C. tropicalis, and C. krusei, are clinically significant. Their biological properties, particularly morphological, cultural, and proteolytic characteristics, vary with environmental changes, influencing pathogenicity[2].

MATERIAL AND METHODS

The taxonomy of microorganisms includes yeast-like fungus belonging to the genus Candida, which has approximately 80 recognised species, of which only a limited number are pathogenic to humans. These encompass C. albicans, C. tropicalis, C. pseudotropicalis, C. krusei, C. guillermondi, C. pelliculosa, and C. parapsilosis[3].

Yeast fungus belonging to the genus Candida are opportunistic pathogenic microorganisms present on the skin and mucous membranes of the human body. These microscopic fungi are found in 30-50% of sputum, urine, feces and skin swabs of healthy people. In the studies of Rebrova RN, it was found that their incidence in the mucous membrane of the human oral cavity increased to 46-52%. Their incidence in the vaginal mucosa of non-pregnant women reached 11-13%, but increased sharply in the third pregnancy and, according to various data, amounted to 29% to 86%. The frequency of occurrence of yeast-like fungus belonging to the genus Candida in feces is 80%, and on intact skin - up to 9%. Candida carriage in clinically healthy people is up to 5%, and in 53.2% of cases of mucous membrane colds[4].

In diagnosing candidiasis and evaluating the degree of dysbiosis in various body biotopes, it is essential to cultivate and quantify yeast-like fungi of the genus Candida. Alterations in environmental conditions of yeast-like fungus belonging to the genus Candida result in modifications of their diverse biological features. In particular, they change the cultural, proteolytic, adhesive and other properties of these fungi. This reduces their production in terms of quality and quantity[5].

The objective of this study was to examine the characteristics of alterations in the cultural and proteolytic properties of yeast-like fungi of the genus Candida in response to diverse conditions.

RESULT AND DISCUSSION

Result

We analysed the morphological, cultural, and proteolytic attributes of the principal yeast-like fungus species, Candida albicans. The identification and differentiation of the isolated microorganism were conducted in accordance with Bergy's methodology. The collection and hospital strains of C. albicans were cultivated three times for comparative analysis[6].

The number of colonies grown from the standard strains of the microorganism (10-6 - 1000 microbial cells in 1 ml, 10-7 - 100 microcells in 12 ml) was quantified. The cultures were obtained from the laboratory of the “National Collection of Microorganisms of Human Infections” of the UZRCCV EMYuKITI. The following were used in the scientific work:

Candida albicans 7 003838, Candida albicans 10 003848, Candida albicans 5 003818, Candida albicans 723 003592[7].

All research was performed at the Urgench branch of the Tashkent Medical Academy and the Institute of Microbiology of the Republic of Uzbekistan utilising standard bacteriological techniques[8].

For statistical processing of the obtained results, the Student and Fisher methods in the modification of Yermolyeva were used. It was carried out using the Excel application programs on a "Pentium - 4" personal computer[9].

Research results. We have developed an expedited approach for evaluating the growth of yeast-like fungus belonging to the genus Candida grown on a rice bran substrate media. We propose to do a qualitative evaluation of the growth parameters of C. albicans colonies cultivated on media according to a specific scale[10].

Level I – satisfactory growth (colonies are characteristic, fully developed, mature, and distinctly visible, exhibiting prolific growth when incubated at 37°C for 18-24 hours, adhering to nomenclatural standards. The morphological, staining, enzymatic, and other biological characteristics of the microbial cultures have remained constant.

Level II – acceptable growth (colonies are often diminutive, exhibiting a delay of 4-6 hours in normal appearance when incubated at 37°C for 18-24 hours; however, the morphological, staining, enzymatic, and other biological characteristics of the microbial cultures remain unchanged);

Level III - minimal growth (colonies are diminutive and visually challenging to identify). Fail to adhere to nomenclature rules; growth is challenging to discern under conditions detectable by conventional means.

Grade IV – no discernible growth (**Table 1**).

Table 1. Evaluation of the growth characteristics of reference cultures of yeast-like fungi of the genus Candida grown on Rice bran aqueous extract(RBAE) -1 and Rice bran aqueous extract (RBAE) -2 on a special scale

Culture, registration number	RBAE - 1				RBAE – 2			
	After 24 hours		After 48 hours		After 24 hours		After 48 hours	
	Concentration							
	104	102	104	102	104	102	104	102
Candida albicans 7 003838	I	V	I	[	[	V	I	I
Candida albicans 10 003848	I	V	I	[	[	V	I	I
Candida albicans 5 003818	I	I	I			[	I	I
Candida albicans 723 003592	I	II	I			[	I	I

RBAE was prepared in 2 versions: in nutrient juice (RBAE -1) and in an isotonic solution of 0.5% NaCl (RBAE -2)[11].

Furthermore, we examined the inherent variability of colony morphology in the standard and hospital strains of Candida albicans grown on RBAE -1 and RBAE-2. In the first passage, the following colony types were found in the Candida albicans population. Morphologically, typical (S form) and atypical – deaf form (K form). With increasing number of passages, the number of S form collection strains increases, due to the decrease

in the number of K form colonies. This indicates that colonies of the deaf form exhibit instability and possess a phenotypic characteristic of variable (**Table 2**).

Table 2. Natural variation in colony morphology in a population of Candida albicans

Passage	Morphological characteristics of colonies					
	Collection strains			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, elevated, lustrous, with smooth margins, and white in colour; on the third day at 37°C, on Sabouraud's medium containing 4% glucose, the diameter measures 3-7 mm. In atypical colonies, all characteristics are uniform, and by the third day, the colony diameter reaches up to 2 mm[12].

When hospital strains were studied, we observed the opposite. As the number of passages increased, the percentage of typical colonies (S appearance) of hospital strains decreased ($r<0.05$), while the K appearance increased. The frequency of these appearances increased 2.3 times in hospital strains at passage III compared to passage I ($r<0.001$).

In all cases, the K expression was the same. The cases we identified show that, firstly, the hospital and reference strains of Candida albicans grew optimally in the proposed medium; secondly, the collection strains are more resistant to repeated passages than the hospital strains[13].

In addition, at passage III, a different morphological type of colony (type R) appeared. These colonies were radially linear, with an uneven surface, protruding from the medium, white in color, up to 4 mm in diameter, and had an incidence of 0.4-0.8%.

The subsequent phase of our research was comparing the alterations in proteolytic activity between collecting and clinical strains of Candida albicans (**Table 3**).

In the collection strains, a higher the proportion of proteolytic activity was observed. observed in the K-form colonies compared to the S-form colonies at passage I ($p<0.05$). The same cultural conditions were maintained for the K-form at passage II. It should be noted that in the collection strains of the S-form at passages II and III, the opposite result was observed - 4±3.3% (in the absence of high and medium proteolytic activity, low proteolytic activity was 96±8%). No such change was observed in the K-form[14].

Table 3. Variation in proteolytic activity of different strains of Candida albicans collection

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 established that in the early passage, the ratio of increased proteolytic activity in the collection strains was 19.3±3% and 10±3.2% higher in the atypical form K than in the typical form S. The same situation was observed in the second passage of these cultures. In the third passage, a negative outcome was noted in the proteolytic activity of the collected strains in form S, however no such specific alteration was detected in form K[15].

Table 4 presents data on proteolytic activity levels (high, average, low) across three passages for two morphological forms: S (typical) and K (atypical).

Table 4. Variation in proteolytic activity of different strains of Candida albicans hospital strains

Passage	Proteolytic activity level					
	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 subsequent studies, we observed a different picture when studying the proteolytic activity of hospital strains. The proteolytic activity of the typical form S was 1.9 times higher than that of the atypical form K. In hospital strains, a negative result for proteolytic activity was detected already at the I passage, in 10.1±4.5% of cases in the S form and 28.9±4.7% in the K form. With increasing quantity the proteolytic activity in the S form colonies consistently diminished throughout the passages high activity was not observed at the III passage.

Discussion

The results of this investigation offer a detailed understanding of the biological variability and proteolytic properties of Candida albicans strains under different culture conditions. The research demonstrated that natural variability in colony morphology is influenced by storage duration and the quantity of passages, with conventional S configurations being dominant in collection strains, while hospital strains exhibited an increased prevalence of the atypical K form over successive passages. Notably, the emergence of the R form in both strain types at the third passage highlights phenotypic plasticity that may be associated with adaptive mechanisms or stress responses.

Furthermore, the evaluation of proteolytic activity revealed significant differences between collection and hospital strains. Collection strains showed a decrease in proteolytic activity by the third passage, with the S form exhibiting only 4% activity, whereas hospital strains demonstrated an earlier reduction in proteolytic activity, particularly with the K form showing a negative result as early as the first passage. These results indicate that hospital strains may undergo rapid phenotypic alterations under in vitro conditions, potentially reflecting their adaptive responses within clinical environments. The proposed use of rice bran aqueous extract (RBAE) as a growth medium was validated, showing consistent colony development and differentiation potential. Overall, the study contributes to the understanding of Candida albicans' phenotypic variability, underlining its clinical significance for diagnostics, antifungal resistance studies, and epidemiological surveillance, as morphological and proteolytic changes may influence pathogenicity, treatment response, and infection outcomes in hospital settings.

CONCLUSION

Fundamental Finding : This study demonstrates that *Candida* yeast-like fungi exhibit satisfactory to moderate growth in aqueous rice bran extract media within 48 hours and possess significant natural heterogeneity in colony morphology, with S, K, and emergent R forms appearing variably depending on strain origin, storage duration, and passage frequency. Additionally, proteolytic activity was shown to decline over successive passages, with a more pronounced reduction observed in hospital strains from the first passage. **Implication :** These findings underscore the diagnostic relevance of monitoring passage numbers and colony morphology in clinical and laboratory settings, while also highlighting the utility of rice bran media as a cost-effective cultivation medium. **Limitation :** However, this study is limited by its focus on a relatively narrow passage window and by not evaluating the full spectrum of enzymatic functions or genetic mechanisms underlying phenotypic shifts. **Future Research :** Further investigations should explore molecular determinants of morphological and proteolytic variability across extended passages and multiple *Candida* species, while also assessing the diagnostic value of rice bran media in diverse clinical microbiology applications.

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