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RESEARCH OF MOLECULAR MASS OF PROTEINS, WHICH OBTAINED BY DIFFERENT TECHNOLOGICAL METHODS OF THE CANDIDA FUNGI CELLS DISINTEGRATION

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Для лікування кандидозу, одного з найпоширеніших захворювань у світі, перспективно розробити субодиничну вакцину на основі білків та полісахаридів клітин грибів Candida albicans та Candida tropicalis.
Мета. *Дослідити за молекулярною масою білки, які отримані з клітин грибів Candida albicans та Candida tropicalis різними технологічними методами окремої дезінтеграції.*
Матеріали та методи. *В дослідженні використовували різні методи дезінтеграції, а саме при дії ультразвуку, розтиранні з абразивним матеріалом та при заморожуванні-розморожуванні. У кожному випадку проводили визначення білків грибів Candida albicans та Candida tropicalis за молекулярною масою за допомогою електрофорезу в 12,5 % поліакриламідному гелі.*
Результати дослідження. *Згідно одержаних результатів було визначено, що в усіх випадках білки були представлені трьома фракціями з молекулярною масою до 10 кДа, молекулярною масою до 75 кДа та молекулярною масою понад 75 кДа, які у кожному випадку майже не відрізнялися кількісним вмістом. Необхідно відзначити значне кількісне домінування фракції білків з молекулярною фракцією до 75 кДа порівняно з іншими фракціями.*
Висновки. *Усі досліджувані методи дезінтеграції, а саме при дії ультразвуку, розтиранні з абразивним матеріалом та при заморожуванні-розморожуванні, забезпечують синхронне відділення однакових фракцій білків клітин грибів Candida albicans та Candida tropicalis*
Ключові слова: *дезінтеграція, технології, ультразвук, розтирання, білки, молекулярна маса, кандидоз, вакцина, антиген*

1. Introduction

Candidiasis is an infectious disease of humans and animals caused by yeast-like fungi of the genus *Candida*, which affects the skin, nails and internal organs and the blood flow system. The number of patients with candidiasis increases every year around the world. *Candida albicans* is the most common pathogen in candidiasis, but also other types of *Candida* mushrooms can cause candidiasis, namely *Candida tropicalis* and others [1].

Many researchers consider as prospective struggle against candidiasis can be development of candida vaccines. Similar studies are actively conducted in many countries of the world: Russia, the USA, Switzerland and others [2].

2. Formulation of the problem in a general way, the relevance of the theme and its connection with important scientific and practical issues

At present, there is no domestic vaccine, which is produced in Ukraine, no foreign vaccine against candidiasis is registered [3] and no research is conducted on the development of candida vaccines.

3. Analysis of recent studies and publications in which a solution of the problem are described and to which the author refers

Researchers offer different variants of candida vaccines: live, killed, subunit and others [4]. The authors consider it promising to investigate subunit vaccines. Subunit vaccines consist of fragments of a microorganism that can provide an adequate immune response. It is known that the main substances in the microcyber fungi of the genus Candida, which possess antigenic properties, are proteins and polysaccharides [5].

4. The field of research considering the general problem, which is described in the article

To obtain a subunit vaccine, it is necessary to break down the pathogen cells and obtain the necessary substances. Destroy the cells can be possible with the use of different methods, which are organized in three groups: physic-mechanical, chemical and enzymatic methods. All processes should be powerful enough to destroy the cell wall, and at the same time soft enough to exclude denaturation or destruction of the target product. And as the cell walls of different microorganisms consist of different polymers, there is no universal method of their destruction [6].

Chemical methods of obtaining proteins of microorganisms have been rejected, since they are based on the processing of chemicals (extraction, hydrolysis) [7]. This is due to the fact that chemicals in contact with antigens significantly weaken the immunogenicity of antigens. Also, it should be noted that methods that require long-term incubation, or methods involving many complex stages, often give a slight protein yield or lead to the denaturation of proteins. Obtaining proteins by physic-mechanical methods of destroying fungi cells is more economical than chemical and chemically-enzymatic. They are performed without the use of expensive and rare reagents or enzyme preparations [6].

For the destruction of the fungus microcircuit, the most affordable, effective and inexpensive physical methods were selected: ultrasound, rubbing cells with solid materials, and freezing-defrosting [8].

Ultrasonic disintegration. It is known that high-frequency vibration of the pounder causes cavitation, that is, the formation of microscopic gas bubbles, which move at high speed near the tip. Bubbles provide the

formation of hydrodynamic forces, which leads to the destruction of cells. Such method of destruction has many advantages, for example, low price and high effectiveness [9].

Freezing- defrosting. Some researchers believe that there is exist an optimal speed of cooling of small cell samples to a low temperature. When this speed is exceeded, part of the cells collapses after recoil. Many researchers believe that cells break down as a result of the formation of intracellular crystals in excess of the critical cooling rate. In the opinion of others, structural changes and cell damage occurs when the temperature is very fast getting below zero.

Grinding with solid materials. This method consists in grinding cells with sand or abrasive powder in the mortar by pounder. It is required that the abrasive parts are as sharp as possible and have the same size as broken cells.

5. Formulation of goals (tasks) of article

Experimental study of the molecular weight of proteins derived from *Candida albicans* and *Candida tropicalis* fungi cells by various technological methods of separate disintegration, namely ultrasound, rubbing with abrasive material and freezing-defrosting.

6. Presentation of the main research material (methods and objects) with the justification of the results

Materials and methods. All studies were conducted in a laminar box under aseptic conditions. *Candida albicans* fungi strain ATCS 1023 and *Candida tropicalis* strain ATC 20336 were separately cultured in test-tubes on Sabouro agar at 25±2 °C for 48 hours and then cells were washed with 10 ml of sterile isotonic 0.9 % solution of sodium chloride. Individually received suspensions of *Candida albicans* and *Candida tropicalis* fungi cells were transferred on Sabur agar mats, then were incubated at 25±2 °C for 6 days after which fungi cells were washed out with 25 ml of sterile isotonic 0.9 % solution of sodium chloride. Microbiological purity of *Candida albicans* and *Candida tropicalis* fungi cells was determined visually and by microscopy. Further, the obtained washings were centrifuged at a rotational speed of 3000 rpm for 15 min. The fungi cells suspension was made up with sterile isotonic 0.9 % solution of sodium chloride to 8x108–8x109 in 1 ml and suspensions were standardized by counting fungi cells in the Goryaev chamber.

In order to disintegrate *Candida albicans* and *Candida tropicalis* fungi cells, several different techniques were used, namely ultrasound, abrasive material, and freezing-defrosting, taking into account all technological aspects, and further analyze of the composition of the proteins by molecular weight.

In the first case, the received biomass of *Candida albicans* and *Candida tropicalis* fungi cells separately in volume of 10 ml of sterile isotonic 0.9 % sodium chloride solution was subjected to ultrasound for destruction of fungal cells on the apparatus of UZUU-21 with predetermined parameters at a purity of 22 kHz, intensity 5 W/cm^2 and at a temperature of 25±2 °C for a further 15 minutes. The temperature of 25±2 °C was constantly monitored and maintained by adding cold water to the surrounding capacity.

In the second case, the received biomass of *Candida albicans* and *Candida tropicalis* fungi cells in a volume of 10 ml was separately rubbed in a mortar with quartz sand for 15 minutes at 25±2 °C, which was added to the fungus biomass in a ratio of 1:1.

In the third case, the received biomass of *Candida albicans* and *Candida tropicalis* fungi cells separately in 10 ml of sterile isotonic 0.9 % sodium chloride solution in a Petri dish was subjected to a fivefold cycle freezing to a temperature -25±2 °C and a defrost up to +25±2 °C

After all destruction processes were completed, for separation of not destroyed cells and cell walls, centrifugation was performed at a speed of 3000 rpm for 15 minutes, then preliminary filtration was carried out on membrane filters with pore diameter of 0.45 μm and sterilizing filtering on diaphragm filters with pore diameter 0 , 22 μm. In each case, the definitions of *Candida albicans* and *Candida tropicalis* protein molecules were determined by electrophoresis in 12.5 % polyacrylamide gel after Lemly [10]. «Broad Range» (BIO-RAD) protein kits were used to detect the molecular weight of the antigens.

To calculate the results, were used statistical methods in biomedical research using Excel.

Research results. As a result of the studies, the composition of *C. albicans* fungus proteins was determined, that was obtained with ultrasound, and was presented with a molecular weight of three fractions: with a molecular weight of up to 10 kDa (11±2 %), a molecular weight of up to 75 kDa (77±7 %) , molecular weight greater than 75 kDa (12±2 %). The composition of the fungal proteins *C. tropicalis* was represented with three fractions: with a molecular weight of up to 10 kDa (10±2 %), a molecular weight of up to 75 kDa (75±7 %), a molecular weight of over 75 kDa (15±3 %). The research results are shown in Fig. 1, 2.

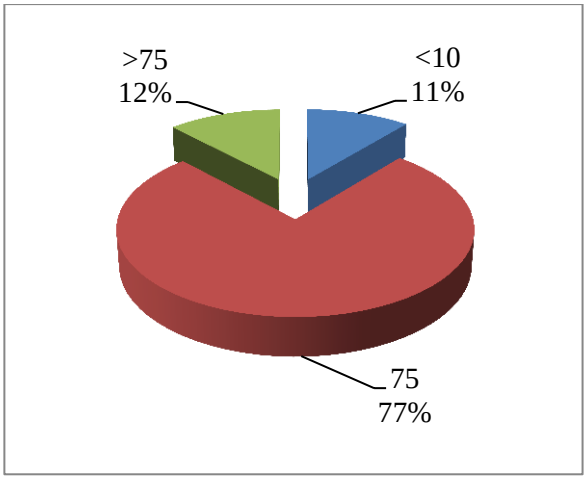

Fig. 1. Characterization of proteins by molecular weight of *C. albicans* fungi cells obtained with ultrasound

As a result of these studies was extracted the composition of the fungus *C. albicans* proteins, which

was obtained after grinding, presented with a molecular weight of three fractions: with a molecular weight of up to 10 kDa (10±2 %), a molecular weight of up to 75 kDa (78±7 %), molecular weight greater than 75 kDa (12±2 %). The composition of the fungal proteins *C. tropicalis* was represented by three fractions: with a molecular weight of up to 10 kDa (10±2 %), a molecular weight of up to 75 kDa (75±7 %), a molecular weight of over 75 kDa (15±3 %). The research results are shown in Fig. 3, 4.

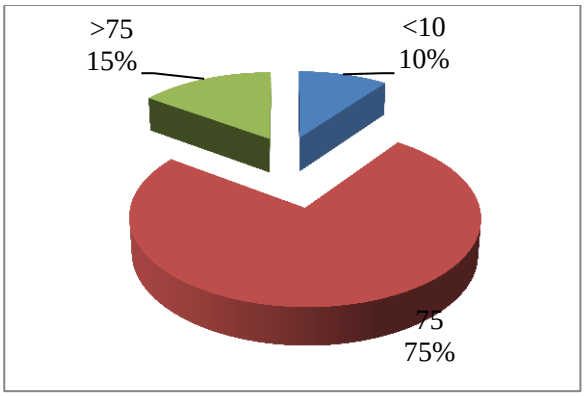

Fig. 2. Characterization of proteins by molecular weight of *C. tropicalis*, fungi cells obtained with ultrasound

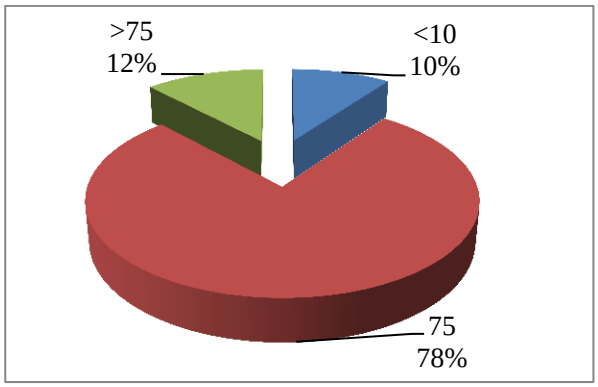

Fig. 3. Characterization of proteins by molecular weight of *C. albicans* fungi cells obtained with grinding

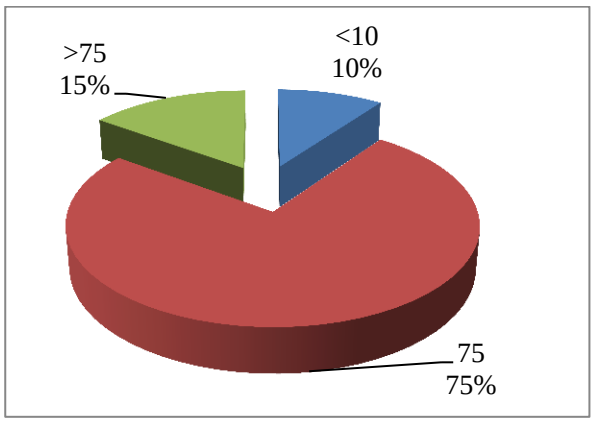

Fig. 4. Characterization of proteins by molecular weight of *C. tropicalis*, fungi cells obtained with grinding

As a result of these studies was extracted the composition of the fungus *C. albicans* proteins, which was obtained by freezing-defrosting presented with a molecular weight of three fractions: with a molecular weight of up to 10 kDa (10±2 %), a molecular weight of up to 75 kDa (73±8 %) , molecular weight greater than 75 kDa (17±3 %). The composition of fungal proteins *C. tropicalis* was represented by three fractions: with a molecular weight of up to 10 kDa (12±2 %), a molecular weight of up to 75 kDa (74±7 %), a molecular weight of over 75 kDa (14±2 %). The research results are shown in Fig. 5, 6.

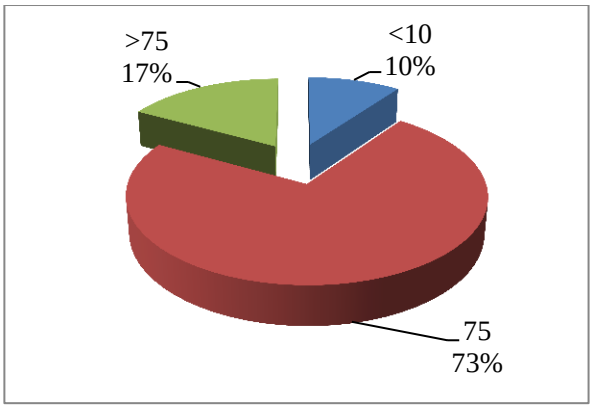

Fig. 5. Characterization of proteins by molecular weight of *C. albicans* obtained with frosting-defrosting

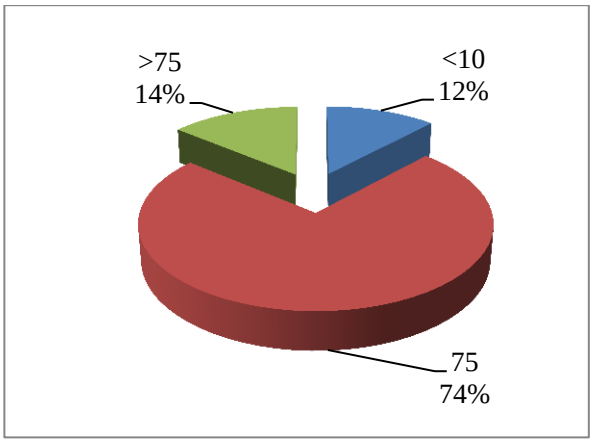

Fig. 6. Characterization of proteins by molecular weight of *C. tropicalis*, fungi cells obtained with frosting-defrosting

As a result of the studies, it was found that all of the test solutions obtained under the action of ultrasound, the methods of grinding and freezing- defrosting *C. albicans* and *C. tropicalis*, have fractions of proteins with a dominant molecular weight of 75 kDa.

Consequently, it can be concluded that all investigated solutions obtained by the action of ultrasound, the methods of grinding and freezing-defrosting *C. albicans* and *C. tropicalis*, have almost the same quantitative and qualitative composition.

However, taking into the account the technological advantages of obtaining solutions of *C. albicans* and *C. tropicalis* fungal proteins using ultrasound, this method of disintegration was chosen for further research.

Also, considering the severity and duration of the methods for disinfecting the *C. albicans* and *C. tropicalis* fungi cells received after grinding and freezing-defrosting, these methods were rejected.

7. Conclusions from the conducted research and prospects for further development of this field

As a result of carried out researches:

1) Three fractions of proteins by the molecular weight of proteins derived from *Candida albicans* and *Candida tropicalis* fungi cells by various technological methods of separate disintegration, namely, by ultrasound, rubbing with abrasive material and freezing-defrosting.

2) In all cases, the proteins were represented by three fractions with a molecular weight of up to 10 kDa, a molecular weight of up to 75 kDa, a molecular weight of over 75 kDa, which in each case practically did not differ in quantitative content.

3) It was established quantitative dominance of the fraction of proteins with molecular weight up to 75 kDa in comparison with other fractions.

4) All investigated methods of disintegration, namely, ultrasound, rubbing with abrasive material and during freezing-defrosting, provide synchronous separation of the same fractions of proteins of fungal cells of *Candida albicans* and *Candida tropicalis*.

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