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BIOTECHNOLOGICAL DESCRIPTION OF TECHNOLOGIES FOR OBTAINING OF ANTIGENS OF *CANDIDA* GENUS FUNGI

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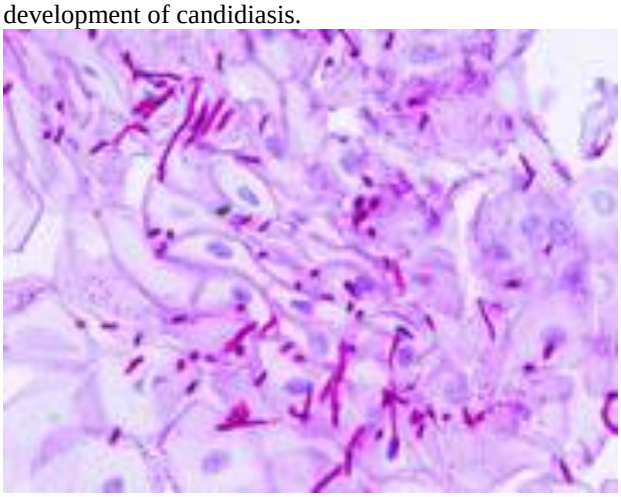
Today mankind is experiencing an epidemic of opportunistic infections, among which mycoses take one of the leading places. Representatives of *Candida* genus are the most often causative agents of mycoses. Candidiasis is an opportunistic mycosis, which proceeds with lesions of the mucous membranes and the skin; disseminated forms, more often with the pulmonary involvement and gastrointestinal disorders, can be in patients with severe immunodeficiency states [1-5]. According to the international research the fungi of this genus are in the forth place in importance among infectious agents in the USA [6-9]. Only coagulase-negative and coagulase-positive staphylococci and enterococci are ahead of them. A similar situation occurs in the countries of the former Soviet Union. From the abovementioned data it follows that trends with candidemia are approximately similar in countries of the Eastern and Western Hemisphere by the epidemic characteristics (including the species ratio of pathogens). Unfortunately, due to the absence of opportunistic mycoses recording in the registration cards of the Ministry of Health in the Russian Federation there are no statistic data on deep mycoses, including candidemias. But, however, according to the sporadic data that include the experience of clinicians – mycologists of the Research Institute of Medical Mycology named after P.N. Kashkin at St-Petersburg Medical Academy of Postgraduate Studies, who consult a number of medical institutions having the patients with invasive fungal infections, in particular fungemia, the number of cases of nosocomial mycoses, candidemias approximately corresponds to the data published in the USA [2, 10-12]. This is largely due to the fact that there are similar risk factors in the world that are the cause of occurrence of candidiases and candidemias (as well as other mycoses and fungemias).

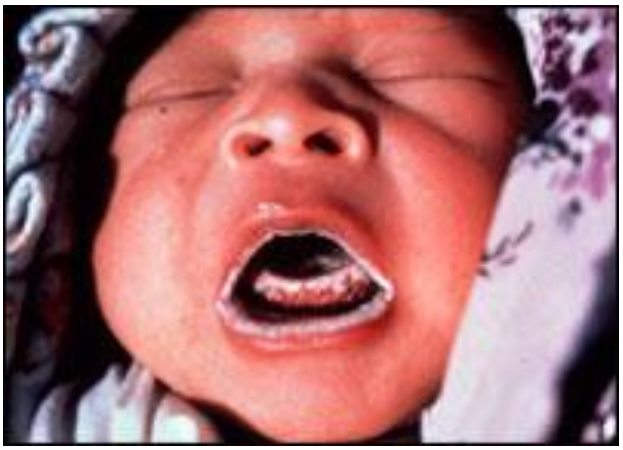
The genus of *Candida* indudes about 163 species, but the main role in the pathological states of the human is played by the limited number of them; they are *C. tropicalis*, *C. parapsilosi*, *C. glabrata*, *C. krusei*, *C. albicans* with a significant predominance of the latter [2-3, 8, 11]. Fungi of the genus Candida easily stained with methylene blue, and Gram-Himozoyu Romanovsky (picture 1).

It should be noted that fungi of this genus are the component of the human normal intestinal and mucous membranes microflora [2, 4-5, 11]. Disturbance of the body's microbial cenosis caused by the inadequate use of drugs of a wide range or changes in the microenvironment stimulates the excessive growth of *Candida* genus fungi [1-2, 9]. Metabolic and hormonal disorders (for example, diabetes mellitus, pregnancy, as well as intake of oral contraceptives) also contribute to development of candidiasis.



Picture 1. World microscopy fungus C. albicans (Gram staining).

Immunodeficiency states and administration of immunodepressants (for example, glucocorticoids) can cause different forms of candidiasis of the skin and mucous membranes or if the conditions, in which a person works or lives, contribute to the development of candidal infections (high humidity, fever, frequent contact with antibiotics or other chemical substances) [2-3, 5, 12]. Candidiasis is characterized by intensive growth of these fungi, displacement of other representatives of the human normal microflora. In disseminated lesions it is formation of foci of necrosis and neutrophilic inflammatory infiltration [2, 4, 8, 11] (picture 2).



Picture 2. Candidiasis

The absence of rapid, sensitive and specific methods for diagnosis of invasive mycoses is a serious drawback in the treatment of such patients, and that is why a lot of persons die before receiving the adequate therapy [2, 8].

Diagnostic and therapeutic problems along with the successful use of antibacterial antibiotics in patients

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with fever and neutropenia became the basis for carrying out the first two randomized studies of antifungal drugs in the 80-ies of the last century. It means empirical therapy of patients with fever and neutropenia when the antibacterial therapy is not effective; it is considered that the most obvious cause of such state of the patients is an undiagnosed fungal infection – usually candidiasis or aspergillosis [2, 4, 8, 11], and delay in treatment may end fatally. Thus, in our time the antifungal therapy is "blind", i.e. without an accurate diagnosis of the fungal infection. The rational empirical antifungal therapy is the treatment of invasive fungal infection at a very early stage of the patient's disease when there is a high risk of such infection [2, 4, 11].

Knowledge of a doctor and the state of a patient play an important role in the choice of the common correct approach concerning the treatment of every patient suffering from candidemia or different forms of invasive disseminated candidiasis. However, the choice of antifungal drugs between Fluconazol, some azoles and polyene amphotericin B is small. Besides, there are a lot of data concerning the loss of *Candida* genus fungi sensitivity to traditional antifungal drugs used for decades [1, 2, 4, 7, 9].

The alternative direction to fight against candidal infection is to develop immunobiological drugs for treatment and prevention of candidiases.

Various vaccines with the antigens of *Candida* genus fungi in their composition belong to these drugs [13-17]

Development of technologies for obtaining antigens of *Candida* genus fungi began from the end of the last century. Whole cells of fungi were the first antigens. They possess the most complete antigenic properties [2, 18-20]. Researchers used filtrates of the culture fluid or filtrates of washings of fungal cells from a dense nutritional medium as antigens. The obtained fungal cells were inactivated by various methods: some researchers used physical methods such as heating, ultrasonic treatment, etc., other researchers applied chemical methods such as formolation, treatment with alcohol, etc.

Later on researchers found more appropriate to use extracts from the destroyed fungal cells for obtaining antigens. Various methods – mechanical, physical, chemical and biological ones were used to disintegrate cells [2, 21-23]. Antigens consisted of fractions of the protein and glycoprotein nature. Each species of *Candida* had antigenic components of the glycoprotein nature that were common with other species and specific for the particular species. Lipid substances in the drugs obtained were presented in minimal quantities and probably in combination with proteins [2, 24, 25, 26].

The interest to mechanical methods of disintegration of *Candida* cells increased even more in connection with the research of the chemical composition and the antigenic structure of the isolated cell walls of fungi [2, 21, 23, 27, 28]. Antigenic drugs were prepared by the physical methods of cell destruction – autoclaving, freezing and thawing in different modes, electrolysis of cells in saline solution, ultrasound; by the chemical methods – by water-alcohol mixture, diethylene glycol, glycerin; surfactants (detergents) were also used [2, 21, 24, 26].

Isolation of antigens with the help of chemical extractants began in the 20-ies. Most researchers tried to obtain antigens by several methods and compare their chemical composition and activity. Some researchers preferred the cold water-alcohol extraction, others used the cell autoclaving with alcohol precipitation, and still others performed the extraction with trichloroacetic acid [2, 23, 25, 27]. The works of a number of researchers by the end of the 50-ies revealed that extracts composed mainly of carbohydrate components – polysaccharides had the greatest activity [2, 21, 22, 27]. The next period of time was devoted to the search of methods for isolation of polysaccharides, their purification, the study of biochemical characteristics and activity.

Before the 60 - 70-s the new data were obtained while studying the structure of the cells of *Candida* genus fungi and the chemical composition of the cell components. It was found that the main part of hydrocarbon components of cells were in the membrane [2, 21, 23, 27]. It was logical to assume that polysaccharides obtained by different impact on the whole cell were isolated from the membrane. Thus, researchers began to study carefully the isolated cell walls.

Some researchers fractionated decontaminated cell walls treating them with 1 N solution of potassium hydroxide [2, 22, 27]. Currently such methods of disintegration as shaking cells with glass beads in disintegrators, crushing the cell mass by a pressure machine, destruction of cells by ultrasound, etc., are used to obtain isolated cell walls [2, 21, 25]. It has been found that in sequential extraction of antigenic substancesfrom *Candida* genus fungi the specificity and activity of isolated drugs decrease with the increase of the hydrolyzing agent rigidity [2, 23, 27].

Some researchers have gone beyond the study of polysaccharides of the cell wall, and investigated extracellular polysaccharides of *Candida* genus fungi, which are present in the culture fluid. Extracellular polysaccharides are presented mainly by mannan, while in the cell wall there are glucan and mannan. It is connected with the solubility of mannan. Extracellular mannans and cell wall mannans do not differ immunochemically, structurally a cell wall mannan is more branched. The comparison of extracellular and intracellular purified mannans and mannan-proteins has shown that a mannan-protein has a greater activity and specificity than a pure mannan considered earlier to be independently responsible for the activity of antigenic drugs [2, 21, 24, 27].

Extracellular polysaccharides – mannan, have less complex structure, low molecular weight and do not form the complex compounds with proteins and lipids. Polysaccharides of the cell wall of *Candida* genus fungi are high-molecular and complexing. Glucan is insoluble in alkali, provides the form and constancy of the cell size, is partially soluble in water. Mannan has a branched structure, is soluble in water, easily isolated by various

methods of extraction of cell walls and the whole cells. Mannan is responsible for the antigen activity and cell specificity of yeast fungi. Their structure is similar in various species of *Candida* genus fungi and some other fungal genuses, and it provides cross reactions. Amino sugar – glucosamine, may play a role of a link in the membrane between proteins and polysaccharides. According to the opinion of many researchers, polysaccharides are in the cell wall as the mannan-glucan-protein, mannan-protein and glucan-protein complex. The attempt of the maximum purification of the *Candida* genus fungi mannan, that is responsible for the antigenic activity, from other biochemical components of the cell, in particular destruction of the complex of polysaccharides with protein, does not increase the activity of antigenic drugs, and decrease their specificity [2, 21-22, 27].

Thus, up to the 80 – 90-s the data were obtained, according to which polysaccharides with *Candida* genus fungi could be divided into 4 groups by their localization in the cell: 1) noncellular polysaccharides released into the environment, 2) soluble or "structural-metabolic" polysaccharides of the cell walls, 3) insoluble or "structural" polysaccharides of the membranes, 4) intracellular or "reserve" polysaccharides [2, 23, 26, 28].

However, all further studies concerning development of vaccines for prevention and treatment of candidal infection in Ukraine stopped since the collapse of the Soviet Union. A difficult and uncertain economic and political situation in the country has negatively impacted on all industries of Ukraine, and only now the work in this field partially begins. It should be noted that the research of the antigens of *Candida* genus fungi and development of vaccines against candidiasis have never stopped abroad, and in many countries on the territory of the former Soviet Union these studies are renewed and carried out actively [15, 20, 22, 25]. It is worth mentioning that currently no domestic vaccine is produced in Ukraine for prevention and treatment of candidal infection and no imported vaccines have been registered.

At first to develop a vaccine against candidiasis it is necessary to analyze the data obtained by previous researchers and to check some of them. It is known that the use of the attenuated cells of *Candida* genus fungi, i.e. live vaccines, is impractical in connection with impossibility of standardization and a possible activation of the causative agent. It is also not appropriate to use products of cell metabolism of *Candida* genus fungi since the biochemical composition of metabolites differs from the composition of the substances contained in the composition of the cells of *Candida* genus fungi, and therefore, they do not provide the necessary immune reactions. When developing a vaccine for prevention and treatment of candidal infection it is expedient to use inactivated cells of *Candida* genus fungi; to develop a dead vaccine. Some components of the cells of *Candida* genus fungi, namely proteins and carbohydrates, can be used for subunit vaccines. For the final choice of the type of the vaccine required it is necessary to develop the technology for their obtaining and to study immune responses.

The technology of development of a dead and subunit vaccine for prevention and treatment of candidal infection supposes cultivation of *Candida* genus fungi, and on their basis development of vaccines is planned. That is why the primary task is to substantiate the technological mode for cultivation of *Candida* genus fungi.

To develop a candidal vaccine it is necessary to determine strains or species of microorganisms, which are the most widespread or main causative agents of candidal infection. According to the literature data in species identification of the fungi taken from the patients with candidal infection it has been found that practically in all cases the fungi of *C. albicans* species are the causative agents of the disease. The second widespread species is *C. tropicalis* occurring, as a rule, in association with *C. albicans.* In some cases *C. parapsilosis*, *C. glabrata* and *C. krusei* have been also determined in association with *C. albicans* or *C. albicans* and *C. tropicalis*) [2, 4, 20, 25]. Thus, conducting the research with the species of *C. albicans* and *C. tropicalis* is promising for development of a combined vaccine [2, 30].

Therefore, summarizing the above said the conclusion can be made that the research concerning development of vaccine against candidiasis is the topical issue of modern pharmacy and medicine.

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To develop the vaccine against candidal infection the various biotechnological methods for obtaining antigens of *Candida* genus fungi have been considered in the article. To obtain antigens for the prevention and treatment of candidiasis researchers use different types and parts of fungi of the genus Candida. Methods of preparation of antigens also vary widely including chemical, physical and physico-chemical techniques. Of all possible variants development and research of a dead and subunit vaccine based on *C. albicans* and *C. tropicalis* fungi that are the main causative agents of candidiasis have been chosen for further study.

Key words: candidiasis; antigen, vaccine, immunity

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ОСВІТЛЕННЯ ТЕХНОЛОГІЙ ОДЕРЖАННЯ АНТИГЕНІВ ГРИБІВ РОДУ *CANDIDA*

Рибалкін М. В.

У даній статті були розглянуті різні біотехнологічні методи одержання антегенів грибів роду *Candida* для розробки вакцини проти кандидозної інфекції. Для одержання антигенів для попередження та лікування кандидамікозу дослідники використовують різні види та частини грибів роду *Candida*. Методи одержання антигенів також широко варіюють включаючи хімічні, фізичні та фізико-хімічні прийоми. З усіх можливих варіантів для подальших досліджень було обрано розробка та дослідження

убитої та суб'єдиничної вакцини на основі грибів *C. albicans* та *C. tropicalis*, які є основними збудниками кандидамікозів.
Ключові слова: кандидамікоз; антиген; вакцина; імунітет

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ОСВЕЩЕНИЕ ТЕХНОЛОГИЙ ПОЛУЧЕНИЯ АНТИГЕНОВ ГРИБОВ РОДА CANDIDA
Рыбалкин Н. В.
В данной статье были рассмотрены различные биотехнологические методы получения антегенив грибов рода Candida для разработки вакцины против кандидозной инфекции. Для получения антигенов для предупреждения и лечения кандидамикоза исследователи используют различные виды и части грибов рода Candida. Методы получения антигенов также широко варьируют включая химические, физические и физико-химические приемы. Из всех возможных вариантов для дальнейших исследований были выбраны разработка и исследование убитой и субъединичной вакцины на основе грибов C. albicans и C. tropicalis , которые являются основными возбудителями кандидамикоз. Оба варианта вакцины предусматривают накопление биомассы грибов на основе которых и планируется разработка вакцин.
Ключевые слова: кандидамикоз; антиген; вакцина; иммунитет