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Research Article

The substantiation of the temperature regime of the freezing-thawing disintegration technology of *C. albicans* fungal cells

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Abstract

In this article, different temperature regimes of the freezing-thawing technology for the disintegration of *C. albicans* fungal cells have been studied. The biomass of *C. albicans* fungal cells in 10 mL of sterile isotonic 0.9% sodium chloride solution in a Petri dish was subjected to destruction using a five-fold freeze-thaw cycle in the following temperature ranges: from (–25 ± 2) °C to (25 ± 2) °C, from (–30 ± 2) °C to (30 ± 2) °C and from (–20 ± 2) °C to (20 ± 2) °C. In order to find the optimal temperature in each case, the content of proteins, polysaccharides, and monosaccharides was determined. According to the results obtained, it has been determined that the temperature regime from (–25 ± 2) °C to (25 ± 2) °C is the most promising since it provides the maximum release of polysaccharides and proteins in the shortest possible time with minimal energy consumption.

Keywords

Candidiasis, disintegration, polysaccharides, proteins, temperature regime

Introduction

Candidiasis is an infectious disease affecting the mucous membranes and the skin. Immunocompromised patients may have disseminated forms that affect the lungs, the gastrointestinal tract, and other organs and tissues (Polesello et al. 2017). Of all types of *Candida* fungi species, *C. albicans* is the most often found in patients with candidiasis. The species *C. albicans* can be either an independent pathogen or in combination with other species of *Candida* fungi (Armstrong-James et al. 2017). Long-term use of the same antifungal medicines for the treatment of candidiasis, such as fluconazole, have led to the loss of sensitivity of *Candida* fungi to these products (Howley et al. 2016; Armstrong-James et al. 2017).

In this regard, many researchers consider the development of vaccines for the prevention and treatment of candidiasis, which act against several species of *Candida* fungi simultaneously, to be a promising direction in the fight against candidiasis (Nami et al. 2019; Tarang et al. 2020). Studies on the development of a candidiasis vaccine are conducted in many countries around the world (Wang et al. 2015; Da Silva et al. 2020). However, no domestic vaccine is currently available in Ukraine, and no foreign candidiasis vaccine has been registered.

Scientists offer different types of vaccines against candidiasis. We believe that the most promising form of vaccine against candidiasis is a subunit vaccine. Subunit vaccines consist of fragments of a microorganism capable of providing an adequate immune response (Edwards et al. 2018;

Piccione et al. 2019). These vaccines do not contain ballast substances that can cause adverse reactions. It is known that the main substances in the cells of the *Candida* genus fungi, which have antigenic properties, are proteins and polysaccharides (Tso et al. 2018).

To obtain a subunit vaccine, it is necessary to destroy the cell of the causative agent of a *Candida* fungus and isolate the required antigenic substances. To destroy (disintegrate) cells, a whole set of methods is used; they can be attributed to three main groups: physico-mechanical, chemical, and enzymatic methods. All procedures should be tough enough to destroy the cell wall, but at the same time soft enough to prevent denaturation or destruction of the antigen. Most animal cells disintegrate relatively easily, but the destruction of plant and bacterial cells often has to deal with certain complications associated with the presence of a cell wall. And since the cell walls of different microorganisms consist of different polymers, there is no universal method of their destruction. Physico-mechanical methods of cell destruction are more economical than chemical and chemical-enzymatic. They are performed without the use of expensive and scarce reagents and enzyme preparations. At the same time, these methods of cell destruction are characterized by a certain non-selectivity: processing can negatively affect the quality of the target substance. A fine adjustment of cell destruction conditions allows some physical methods to isolate one fraction of intracellular contents.

The most affordable, effective, and inexpensive physical methods of cell destruction were selected for the destruction of fungal cells: ultrasound, grinding of cells with solid materials, and freezing-thawing. In this article, different temperature regimes of the freezing-thawing technology for the disintegration of *C. albicans* fungal cells have been studied.

Many researchers believe that there is an optimal cooling rate of small cell samples to a low temperature for the destruction of cells of microorganisms during freezing-thawing. When this speed is exceeded, some cells are destroyed after thawing. Some researchers think that cells are destroyed as a result of the formation of intracellular crystals when the critical cooling rate is exceeded. According to others, structural changes and cell damage occur with very rapid cooling to sub-zero temperatures. Subsequently, the studies on the immunization effectiveness with the antigenic substances obtained were conducted (Rybalkin et al. 2020a, b).

The aim of this work was to experimentally substantiate the temperature increase of the freezing-thawing technology for disintegrating *C. albicans* fungal cells.

Materials and methods

All studies were performed in a laminar flow box in aseptic conditions. *C. albicans* cells of SSM 335-867 strain were cultivated according to the scheme in test tubes on Sabouraud agar at (25 ± 2) °C for 48 h; the fungal cells were washed with 10 mL of sterile isotonic 0.9% sodium chloride solution. The suspensions of *C. albicans* fungal cells obtained were transferred to mattresses with Sabouraud agar; they were incubated at (25 ± 2) °C for 6 days, and the fungal cells were washed with 25 mL of sterile isotonic 0.9% sodium chloride solution. The microbiological purity of the suspension of *C. albicans* fungal cell was determined visually and by microscopy. The resulting washings were then centrifuged at 3000 rpm for 10 min. The precipitate of fungal cells obtained was diluted with sterile isotonic 0.9% sodium chloride solution to (8.5–9) $\times 10^8$ in 1 mL, and the suspensions were standardized by counting fungal cells in the Goryaev chamber.

To determine the optimal temperature for the disintegration of *C. albicans* fungal cells during freezing-thawing, the studies were performed at the temperature ranges from (–25 ± 2) °C to (25 ± 2) °C, from (–30 ± 2) °C to (30 ± 2) °C, from (–20 ± 2) °C to (20 ± 2) °C and with the subsequent analysis of the composition of the substances obtained. The biomass of *C. albicans* fungal cells in 10 mL of sterile isotonic 0.9% sodium chloride solution in a Petri dish was subjected to destruction using a five-fold freeze-thaw cycle in the specified temperature ranges. After completion of all destruction processes, centrifugation was performed at a speed of 3000 rpm for 10 min to separate intact cells and cell walls, then pre-filtration on membrane filters with a pore diameter of 0.45 μm and sterilizing filtration on membrane filters with a pore diameter of 0.22 μm were carried out. In each case, the content of polysaccharides and proteins were determined.

The protein determination was performed according to the State Pharmacopoeia of Ukraine (SPhU). Polysaccharides were determined by the reaction with phenol and sulfuric acid. The reaction proceeds with the formation of red-brown colored compounds. 1.0 mL of the polysaccharide solution was transferred to a test tube; 1.0 mL of 5.0% phenol solution and 5.0 mL of the concentrated sulfuric acid were sequentially added. The reaction solution was heated; in a few seconds, a red-brown color appeared. Chromatographic studies of monosaccharides were performed by paper chromatography according to the SPhU. To calculate the results, statistical methods of biomedical research using Excel software were applied.

Results and discussion

The results of studying the composition of the solutions indicate that the solutions obtained at temperatures from (–25 ± 2) °C to (25 ± 2) °C and from (–30 ± 2) °C up to (30 ± 2) °C during freezing-thawing the biomass of *C. albicans* fungal cells contained the largest amount of proteins and polysaccharides. It is likely that in these temperature ranges there is a release of active substances from all layers of *Candida* fungal cells. Fig. 1 shows the protein concentration in the solutions of *C. albicans* obtained depending on the disintegration temperature regime.

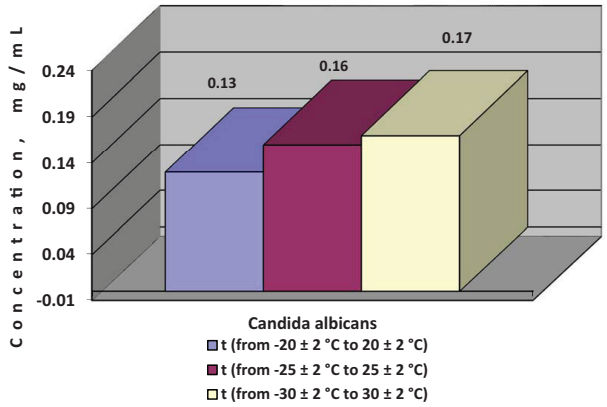

Figure 1. The concentration of proteins depending on the temperature regime of *C. albicans* disintegration, n = 10, P < 0.05.

Fig. 2 shows the indicators of the polysaccharide concentration in the solutions of *C. albicans* fungi obtained depending on the disintegration temperature regime.

The solution obtained at a temperature of (–20 ± 2) °C to (20 ± 2) °C during freezing-thawing of the biomass of fungal cells *C. albicans* fungal cells contained less polysaccharides and proteins.

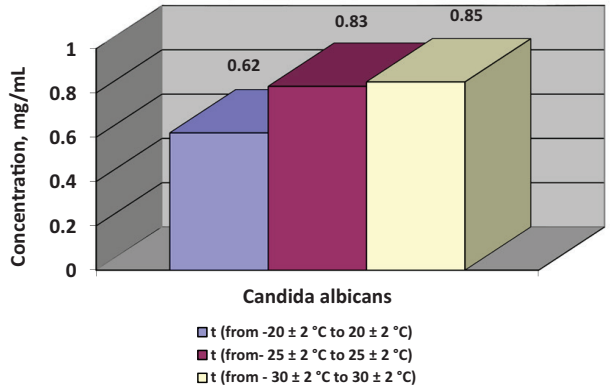

Figure 2. The concentration of polysaccharides depending on the temperature regime of *C. albicans* disintegration, n = 10, P < 0.05.

Polysaccharides of *C. albicans* are represented by several monosaccharides. The qualitative determination and quantitative comparison of monosaccharides were performed by the intensity of the spot coloration during paper chromatography.

Polysaccharides of solutions obtained at temperatures from (–25 ± 2) °C to (25 ± 2) °C and from (–30 ± 2) °C to (30 ± 2) °C when freezing-thawing the biomass of *C. albicans* fungal cells were represented by monosaccharides: mannose, glucose, and two unidentified monosaccharides. Polysaccharides of the solution obtained at a temperature of (–20 ± 2) °C to (20 ± 2) °C during freezing-thawing the biomass of *C. albicans* fungal cells were represented by the same spectrum of detected monosaccharides, but the spots were less saturated, indicating smaller amount of monosaccharides in this solution. Fig. 3 shows the monosaccharide composition of *C. albicans* fungal polysaccharides during freezing-thawing at all temperature modes.

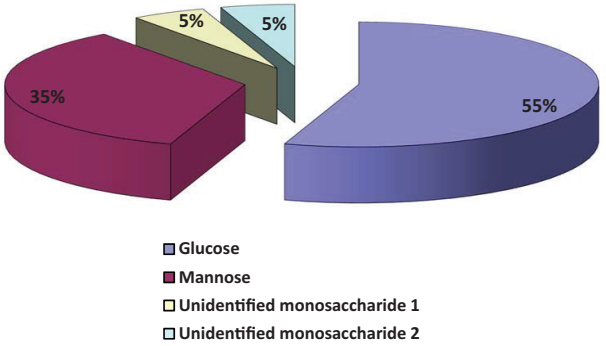

Figure 3. The monosaccharide composition of *C. albicans* fungal polysaccharides during freezing-thawing at all temperatures, n = 10, P < 0.05.

According to the research results, it has been found that under all temperature conditions of disintegration proposed during freezing-thawing of *C. albicans* fungal cells there is a release of polysaccharides possessing the same monosaccharide composition. However, the largest amount of proteins and polysaccharides was obtained using an increase in temperature from (–25 ± 2) °C to (25 ± 2) °C and from (–30 ± 2) °C to (30 ± 2) °C.

To substantiate the optimal method of disintegration during freezing-thawing of *C. albicans* fungal cells, in addition to comparing the yield of proteins and polysaccharides in each specific case, it is necessary to analyze the technological aspects of each method. Disintegration of fungal cells at a temperature from (–30 ± 2) °C to (30 ± 2) °C is characterized by higher resource consumption. As for the disintegration of fungal cells at a temperature from (–25 ± 2) °C to (25 ± 2) °C, it can be concluded that it is more rational and economical as it requires less resources. Since in both cases the amount of proteins and polysaccharides is approximately the same, it is rational to choose a more economical option for further research, i.e. the disintegration of *C. albicans* fungal cells at a temperature from (–25 ± 2) °C to (25 ± 2) °C.

Conclusion

Based on the studies conducted on substantiation of the freezing-thawing temperature regime for disintegration of *C. albicans* fungal cells, taking into account the concentration of proteins, polysaccharides, and monosaccharide composition, it can be concluded that the optimal temperature is from (–25 ± 2) °C to (25 ± 2) °C. This temperature provides a high yield of proteins and polysaccharides. It is economical since it requires low energy consumption.

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