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Вплив режимів заморожування-відтавання тканини плаценти на склад і біологічну активність її кріоекстрактів

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Effect of Placental Tissue Freeze-Thawing Modes on Composition and Biological Activity of Its Cryoextracts

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Application of low temperatures for processing biological materials, depending on the mode of freeze-thawing, provides for higher production of biologically active substances within cryoextracts. Substantiation of the usage of cryotechnologies for isolating biologically active substances from fetoplacental tissues is one of the current problems of cryobiology.

Cryoextracts (PCE) were obtained from rat placental homogenates following single (–20°C) – mode 1, two-stage (–20, –196°C) – mode 2, and three-stage (–20, –196, –196°C) – mode 3 – freeze-thawing. Protein-peptide content of cryoextracts was characterized using size-exclusion chromatography. PCE biological activity was evaluated *in vitro* by the phagocytic activity of rat blood neutrophile granulocytes (NG) during their incubation with a *Staphylococcus aureus* (2×10^9 cells/ml) inactivated culture within 45 and 120 min for estimated PCE concentrations in the incubation medium: 1.6; 3.1; 6 mg/ml. NG consumption was evaluated by a phagocytic index PI (average amount of bacteria, consumed by a phagocytosed cell). A factor of phagocytosis completion (ratio of PI45 min to PI120 min, PI45/PI120) was used for evaluating NG digesting ability.

Analysis of gel chromatograms showed that the volume fraction of proteins with molecular masses (Mm) from 20 to 150 kDa in placental cryoextracts depending on the mode of isolation comprised: PCE-1 – 80.28%; PCE-2 – 79.22%; PCE-3 – 72.78%, while total concentrations of proteins in cryoextracts PCE-1, PCE-2 and PCE-3 were 23.81; 22.92 and 25.01 mg/ml, respectively. The volume fraction of substances with Mm ranging from 4 to 12 kDa, containing low-molecular proteins and peptides, also depended on the mode of isolation and comprised 19.60% – for PCE-1, 20.78% – for PCE-2, and 27.04% – for PCE-3. However, mode 3 produced a higher amount (by 30%) of the above substances as compared with mode 1. A study of biological activity showed that NG incubation with PCE independent of concentrations and modes of isolation did not result in a significant increase in the number of phagocytosed neutrophils. However, all the PCE samples demonstrated a dose-dependent increase in NG consumption following 45-min incubation as compared with the control. Analysis of a NG digestive ability testified to their higher activation following incubation with PCE-3.

Application of a three-stage cycle of placental homogenate freeze-thawing for isolation of cryoextracts from them provided for higher production of total proteins and increased yield of low-molecular compounds of a protein-peptide nature. Placental cryoextracts, independently of the mode of isolation, provided for an increase in NG consuming activity following incubation *in vitro*.

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Антагоністична активність іммобілізованих симбіотиків після ліофілізації та зберігання за різних температур

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Antagonistic Activity of Immobilized Symbiotics After Lyophilization and Storage at Different Temperatures

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Immobilized symbiotics are becoming increasingly common in the global market of functional foods. In this regard, there was a problem with developing effective and affordable technologies for the storage of such products. The most common methods of storage of medical, veterinary drugs and food products containing live microorganisms are storage at low temperatures, lyophilization, vacuum drying. The use of these methods for the storage of immobilized symbiotics is under development.

Therapeutic and prophylactic action of probiotic strains of microorganisms, which are a part of the symbiotic microorganisms, mostly depends on their antagonistic activity.

The aim of the study was to investigate the effect of lyophilization followed by storage at different temperatures on the antagonistic activity of immobilized symbiotics.

The objects of the study were probiotic strains *Lactobacillus bulgaricus, Bifidobacterium longum, Bifidobacterium bifidum,* immobilized in granules of 1% alginate gel with an admixture of 10% prebiotic FOS (a mixture of oligomers, which include fructose and glucose). After lyophilization, the granules were stored for a year at 30, 4, –20, –40, –75...–80°C. During storage, the samples of the granules were dissolved in a 4% EDTA solution.

Cells were washed and suspended in MPC-5 liquid culture medium to obtain a concentration of 10^8 cells/ml. The antagonistic activity of probiotic strains was studied by the methods of delayed antagonism and two-layer medium with the determination of MICA. The cell concentration was determined by Koch's method.

It was found that storage at 30 to –40°C led to a death of a part of the lyophilized cells. The number of dead cells augmented with increasing a storage temperature. At –75…–80°C the lyophilized cells did not die during a year.

The processes of lyophilization and storage at all studied temperatures did not affect the spectra and the severity of the antagonistic activity of the probiotic strains *L. bulgaricus, B. longum, B. bifidum* immobilized in alginate gel with an admixture of FOS prebiotic. Areas of growth retardation of pathogenic and opportunistic microorganisms and MICA did not differ from the control parameters.