Probl Cryobiol Cryomed 2023; 33(4):289
https://doi.org/10.15407/cryo33.04.289

Порівняльний аналіз чутливості еритроцитів собаки та людини до зміни осмотичних умов середовища

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Comparative Analysis of Sensitivity of Canine and Human Erythrocytes to Changes in Osmotic Conditions of the Medium

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Currently, the long-term storage of canine erythrocytes is the least developed approach if compared to that of human ones, although the development of veterinary medicine predetermines introduction into practice of cryopreserved animal cells. Based on this, the researches aimed to study the sensitivity of canine erythrocytes to the effect of different cryoinjuries, implemented at various stages of cryopreservation, are necessary for the further development of conditions and media for their storage.

Research aim was to study the response of canine erythrocytes to changes in the osmotic media conditions in comparison with human cells.

Hypotonic stress (HS) of erythrocytes was initiated by transferring the aliquots of cell suspension to hypotonic media containing NaCl (40–120 mmol/L). To perform hypertonic shock (HSH), aliquots of the suspension were placed in a solution containing NaCl (1.0–4.0 mol/L). Temperature was 37 or 0°C, and the incubation time was 5 min. The hemoglobin content in the supernatant was determined spectrophotometrically (λ = 543 nm).

Studying the sensitivity of canine and human erythrocytes to the effect of HS, the dependencies of erythrocytes hemolysis on the concentration of NaCl were obtained, which in general have the same character, but possess some specific features. Thus, in the medium containing 40 mmol/L NaCl, the level of hemolysis of canine erythrocytes was about 80%, for human ones this was 100%. At 37°C, there were practically no differences in the values of threshold concentration (70 mmol/L NaCl) and osmotic fragility index (~55 mmol/L NaCl) for canine and human erythrocytes. At 0°C, there was a rise in the threshold concentration values up to 80 mmol/L NaCl for cells of both species and an increase in the osmotic fragility index values up to 60 and 71 mmol/L NaCl for canine and human erythrocytes, respectively. When investigating the hypertonic hemolysis level of canine and human erythrocytes, it was established that within a wide range of nonphysiological concentrations of NaCl, cells were not damaged, but began to lyse (hemolysis above 10%) in the medium containing 2.75 mol/L NaCl. In a highly concentrated saline (4.0 mol/l NaCl), differences in the sensitivity of cells to the effect of HSH were observed at both temperatures. Canine erythrocytes were more stable under the conditions of HSH. Comparative analysis showed that at 37°C the hemolysis level of human erythrocytes exceeded the one of canine erythrocytes by 1.7 times, at 0°C this was by 2.1 times higher. Therefore, the temperature-osmotic characteristics of canine erythrocytes determined in this research and the results of analyzed publications indicate the inexpediency of direct application of cryopreservation conditions, developed for human erythrocytes to the canine cells.

Probl Cryobiol Cryomed 2023; 33(4):289
https://doi.org/10.15407/cryo33.04.289a

Вплив солоності на стійкість рослинних клітин до низьких та наднизьких температур на прикладі клітин мікроводорості *Dunaliella salina*

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The Effect of Salinity on the Resistance of Plant Cells to Low and Ultra-Low Temperatures on the Example of *Dunaliella salina* Microalgae Cells

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The need to conserve plant genetic resources through cryopreservation techniques to mitigate the effects of climate change such as extinction of certain plant species cannot be underestimated. Studying the adaptive mechanisms that occur in plant cells in response to stress can increase their resistance to cryopreservation. The diverse stress factors that plants have to face often activate cellular responses such as the production of stress proteins, upregulation of the antioxidant machinery, and accumulation of protective substances.

The aim of the work was to study the effect of salinity of the cultivation medium on the synthesis of valuable metabolites and the resistance of *Dunaliella salina* microalgae cells to low and ultralow temperatures.

In our work, we used *Dunaliella salina* cell culture, which was grown under stress conditions to increase the yield of valuable products, in particular, to increase the amount of lipid globules containing carotenoids. Microalgae were cultivated in Ramaraj medium containing 1.5 M, 3 M, and 4 M sodium chloride. We assumed that an increase in the salinity of the medium would increase the accumulation of valuable metabolites, which would increase the resistance of microalgae to low and ultralow temperatures.

The study of chlorophyll autofluorescence and Nail Red dye fluorescence in microalgae cells using a LSM-510 Meta laser scanning microscope (Carl Zeiss, Germany) showed that with an increase in the content of sodium chloride in the cultivation medium, the intensity of chlorophyll autofluorescence decreased and Nail Red fluorescence increased.

Cell cooling to temperatures of −10°C, −40°C and to −40°C with further immersion in liquid nitrogen (−196°C) showed that the resistance of microalgae cells to low temperatures increased with an increase in the content of sodium chloride in the medium. Thus, the concentration of visually undisrupted cells upon cooling to −10°C did not decrease in comparison with the control in all the studied samples. A significant decrease in cell concentration was observed when the studied samples were cooled to temperatures of −40 and −196°C. At the same time, a direct relationship was established between the amount of sodium chloride in the culture medium and the concentration of cells after freezing/thawing.

The results indicate that an increase in the salinity of the medium for cultivating microalgae *Dunaliella salina* augments the concentration of valuable metabolites, which makes it possible to improve the safety of cells after freeze-thawing. The results can be used to develop the methods for cryopreservation of various types of plant objects and to increase the cell preservation after freeze-thawing.