Preservation rate of microorganisms after freezing down to -196° C in non-covalent gels

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INTRODUCTION

Current medical technologies, veterinary medicine, food industry there often apply the drugs containing the live microbial cells and their metabolites. For long-term storage of commercial forms of these drugs freeze-drying, thermal drying and storage at low temperatures are used. Those preparations are stored under low temperatures in different protective media. They reduce the damaging effect of physical and chemical factors, the development of which is related to crystallization, i.e. re-crystallization of water during cooling and thawing.

In contrast to classical cryoprotectants solutions, gels are polymer-solvent system. Polymeric grid of the system is stabilized in space throughout the volume by intermolecular bonds. Crystallization processes and damaging factors in the gels are expected to be less pronounced. Gels are used as matrixes in the manufacturing of the immobilized preparations for oral application and in the designing of drug delivery systems (DDS).

It is desirable to use gels with high cryoprotective properties as matrixes.

AIM

The aim of our study was to assess viability of the *Saccharomyces boulardii* yeast cells and *Escherichia coli* M-17 bacteria after freezing down to -196°C in non-covalent gels.

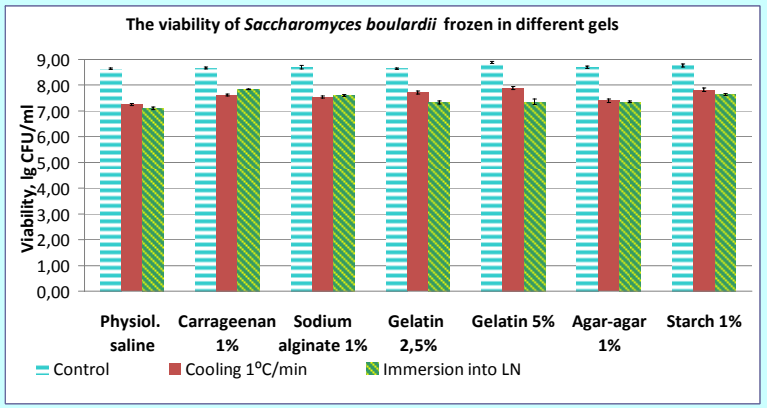
MATERIALS AND METHODS

The objects of study: yeasts *Saccharomyces boulardii*, bacteria *Escherichia coli* M-17. **Gels**: kappa-carrageenan (1%), alginate (1%), gelatin (2.5; 5%), agar (1%), starch (1%). During a controlled freezing, the samples were cooled up to to - 40°C with the cooling rate of 1 or 20°C/min and then were immersed into liquid nitrogen (-196°C). Rapid cooling was carried out by immersing of samples into liquid nitrogen. Viability of the microorganisms was determined by the number of macrocolonies formed on the agar media (Miller J., 1972). The immobilization of microbial cells in sodium alginate gel beads and kappa-carrageenan was performed by methods described by Tsen J.H. et al. (2007).

RESULTS

In the first part of the experiment the microbial cells were frozen in a cylindrical gel blocks (h=20 mm, d=15 mm). *S.boulardii* cells suspended in physiological saline and *E.coli* M-17 cells in growth medium M9 (Miller J., 1972) were used as controls. It was found that the number of viable *S.boulardii* cells, frozen in the gel blocks was higher as compared to cell viability in control. Cooling regimens did not affect the viability of yeast cells in the gel blocks. In the second part of the experiment after freezing of *S.boulardii* cells immobilized in kappa-carrageenan and sodium alginate beads, the similar results were obtained.

When freezing gel blocks by immersion them into liquid nitrogen the number of viable *E.coli* M-17 cells did not significantly change in all samples except 1% agar. After cooling at 20 °C/min the number of viable cells decreased in all samples. After cooling at 1 °C/min the number of viable bacteria decreased in control blocks of alginate and agar. Overall, these declines were small - from 0.1 to 0.19 lg CFU/ml. After freezing of the cells immobilized in kappa-carrageenan and alginate gel granules the similar results were obtained.



Viability of *E. coli M-17* cells after freezing in gel blocks (different regimens)

Cryopreservation medium	Viability, (M±m) lg CFU/ml			
	Controls	Cryopreserved cells		
		1 °C/min	20 °C/min	Immersion into LN
M9	9,53±0,03	9,25±0,04*	9,44±0,02*	9,50±0,03
1% sodium alginate	9,62±0,04	9,43±0,04*	9,52±0,03*	9,62±0,05
1% kappa-carrageenan	9,60±0,05	9,54±0,03	9,44±0,04*	9,61±0,01
5% gelatin	9,54±0,04	9,50±0,03	9,44±0,02*	9,51±0,05
1% agar	9,64±0,03	9,20±0,05*	9,57±0,03*	9,48±0,04*
1% starch	9,61±0,03	9,56±0,02	9,45±0,01*	9,60±0,03

* - significant compared to controls (p< 0,05)

CONCLUSIONS

1. Non-covalent gels of polysaccharides of organic origin have cryoprotective effect during freezing *S.boulardii* and *E.coli* M-17 cells up to -196 °C temperature.
2. For long-term storage preparations of microbial cells immobilized in constructions of polysaccharide gels cryopreservation can be used.