Viability of Cryopreserved Probiotic Strains of Microorganisms Immobilized in Alginate Gel Supplemented with Disaccharides

I.V. Petrov, I.A. Buriak, Ya.O. Cherkashina

Institute for Problems of Cryobiology and Cryomedicine of NAS of Ukraine, Kharkiv, Ukraine

Probiotics are widely used in medicine and veterinary medicine as treatment and prophylaxis, as functional nutrition components, as well as in agriculture for feed mixture production. Probiotics immobilized in gel carriers are getting more popular. Technologies of long-term storage of probiotics immobilized in gel carriers have not been developed yet. Cryopreservation is one of the most effective methods of long-term storage of different microorganisms.

Research purpose was to study the effects of concentrations of sodium alginate and disaccharides (sucrose and lactose) on the viability of immobilized probiotic strains of microorganisms. The test subjects were Lactobacillus acidophilus, Escherichia coli M-17 and Saccharomyces cerevisiae obtained from the Ukrainian collection of microorganisms of the D.K. Zabolotny Institute of Microbiology and Virology of NAS of Ukraine. Granules of sodium alginate gel were prepared by ionotropic gelation. The viability of microorganisms was assessed by colony formation in Petri dishes. Phase transitions during freeze-thawing of the solutions of sodium alginate, disaccharides and their mixtures were investigated by thermo-mechanical analysis on a strain-gauge dilatometer for cryobiological research (IPC&C of the NAS of Ukraine). Samples were frozen by two programs: slow cooling at a rate of 1 deg/min down to −40°C followed by immersion in liquid nitrogen and rapid cooling via immersion into liquid nitrogen.

It was found that during freeze-thawing the sodium alginate concentration within a range of 0.5–2% in gel granules did not significantly affect the viability of immobilized microorganisms. Supplementation of 1% alginate gel with sucrose or lactose in concentrations of 5, 10, 15, or 20% increased the viability of immobilized probiotics. The highest viability was achieved in gel granules containing 10% - 20% disaccharides. Cooling modes also significantly affected the viability of immobilized probiotics. The maximum viability was observed for all the samples after slow cooling. The thermomechanical (TM) curves of 1% and 2% sodium alginate solutions did not differ. The TM curves of disaccharide solutions had intrinsic bends within the corresponding temperature intervals. There were bends intrinsic to each component (1% alginate sodium + 5% sucrose or lactose) on the TM curve of the supplemented carrier.

The results allow us to recommend supplementation of the carrier with disaccharides in the development of cryopreservation protocols for these probiotics immobilized in sodium alginate gel.