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Збереженість іммобілізованих симбіотиків після кріоконсервування та інкубації в умовах імітаційної моделі травлення людини

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Viability of Immobilized Symbiotics After Cryopreservation and Incubation in Human Digestion Model

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The protection of probiotic drugs against damaging effects of gastrointestinal tract protective barriers is a required condition for efficient probiotic therapy. In this regard, the studies on designing the novel dosage forms of probiotics and symbiotics, immobilized in gel carriers are currently in progress. Low temperatures and lyophilization are most frequently applied for a long-term storage of probiotics. The use of these technologies to preserve the immobilized symbiotics is under development.

The research aim was to study the impact of freezing down to –196°C and subsequent incubation in the media, simulating gastric and duodenal juices on the viability of probiotic bacteria, combined with prebiotics and immobilized in alginate gel.

Symbiotics (a mixture of bacteria with oligosaccharides) were immobilized in granules of 1% alginate gel by ionotropic gelation. The samples of bacteria, immobilized in 1% alginate gel free of oligosaccharides, served as the control. Gel granules were cooled down to –40°C with 1 deg/min rate, then immersed into liquid nitrogen. The samples were thawed in a water bath at 37°C. The media, simulating gastric (pH 2.0–2.2) and duodenal (pH 7.0–7.2) juices contained Acidin-pepsin and Panzynorm forte 20 000, respectively medium 1 and 2.

It was found out that after freezing in oligosaccharide-free alginate gel the bacterial viability made 80%, and 90–95% for oligosaccharide-contained one. After heating and subsequent incubation for 4 hrs in the medium 1, the number of viable bacteria decreased by 17 and 10%, respectively. After incubation in the medium 2, it reduced by 10 and 6%, respectively. After alternating incubation in the media 1 and then 2, the number of dead cells increased, while the viability in the control samples was lower as well.

Our findings testify to a protective effect of oligosaccharides within the gel, both during freezing and incubation in the media 1 and 2.

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Відновлення популяцій ценобіонтів *Bifidobacterium spp.*, *Lactobacillus spp.* після терапії експериментального дисбіозу іммобілізованим пробіотиком *Escherichia coli* М-17, що зберігався за низьких температур

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Recovery of Cenobiont Populations of *Bifidobacterium spp.* and *Lactobacillus spp.* after Treatment of Experimental Dysbiosis with Immobilized Probiotic *Escherichia coli* M-17 Stored at Low Temperatures

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The latest-generation probiotic drugs are immobilized probiotics, which are widespread in medicine, veterinary medicine and food industry. Methods for their long-term storage at low temperatures are under development.

Our purpose was to investigate the effect of low temperature storage on the ability of the probiotic strain *E. coli* M-17 immobilized in unmodified and modified alginate gels to restore eubiotics of the intestinal microbiocenosis.

E. coli M-17 bacteria were immobilized by ionotropic gelation in granules of 1% alginate and modified gels. The 1% alginate gel was modified by adding 20% sucrose or protective medium, containing sucrose, skimmed milk and lactose. Ten gel granules contained 1 therapeutic dose of *E. coli* M-17 for mice which was 10^5 CFU. Samples were stored at –80 and –196°C for 1 year. Experimental intestinal dysbiosis in mice was induced by 3-day intragastric administration of ampicillin (5 mg) and metronidazole (3 mg). The quantitative and qualitative composition of intestinal mucosa-associated microflora of the colon were determined by conventional methods. Cenobionts *Bifidobacterium spp*. and *Lactobacillus spp.* were used as markers of the intestinal microbiocenosis.

After 1-year storage under low temperatures, the probiotic immobilized in modified gel provided a recovery of the cenobiont concentrations of *Bifidobacterium spp*. and *Lactobacillus spp.* in parietal mucin of the colon in mice. The therapeutic effect of the probiotic immobilized in unmodified gel was less pronounced compared to the one immobilized in the modified gel.

Thus, concentration of *Bifidobacterium spp*. before intestinal dysbiosis induction was 7.4 lg CFU/g; right after the induction of dysbiosis it was 2.0 lg CFU/g; and 20 days later it was 4.0 lg CFU/g. The concentrations of *Lactobacillus spp.* were 8.1, 2.3 and 3.8 lg CFU/g, respectively. Ten days after the completion of therapy with probiotic immobilized in unmodified gel, the concentrations of cenobionts were 6.2 and 7.2 lg CFU/g (probiotic was stored at –80°C) or 6.4 and 7.2 lg CFU/g (probiotic was stored at –196°C). In all the groups of animals injected with probiotic immobilized in modified gel and stored at –80 or at –196°C, the concentration of *Bifidobacterium spp*. was 7.2–7.7 lg CFU/g, and the one of *Lactobacillus spp.* made 8.0-8.5 lg CFU/g.