Biological properties of probiotic microorganisms after immobilization on carbon-containing sorbents and low temperature storage

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INTRODUCTION

In some cases, there is no effect of probiotics usage during the correction of dysbiotic changes in the gut. This is due to the death of some cells in the gastrointestinal tract and probiotic bioincompatibility with patient's microflora. Therefore, new drug forms including immobilized probiotic formulations are created.

AIM

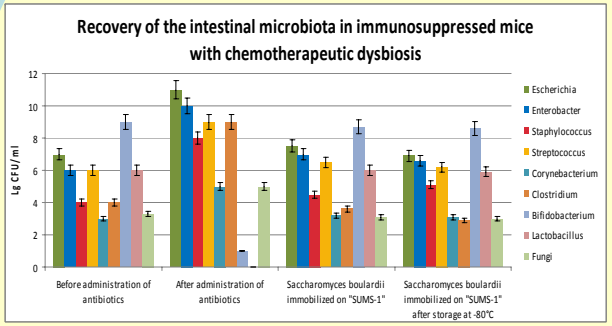
The aim of our research was to investigate the biological properties of probiotics after immobilization on enterosorbents and low temperature storage.

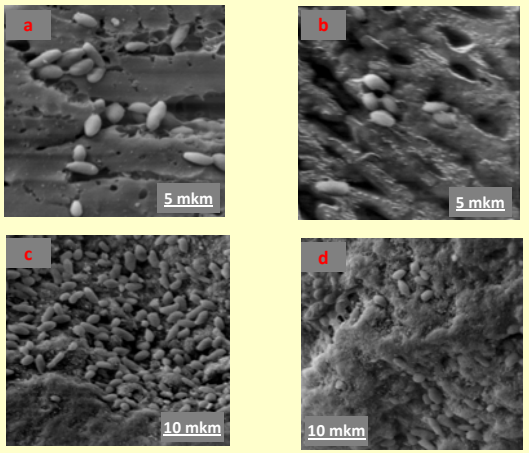
MATERIALS AND METHODS

Probiotics: *Saccharomyces boulardii*, *Bifidobacterium bifidum*, *Lactobacillus delbrueckii subsp. bulgaricus*. **Enterosorbents**: "Sorbex" (Ecosorb, Ukraine), SUMS-1 (Novosibkhimpharm, RF) – both contain carbon.

Immobilization method: shaking of microorganisms' cells suspensions with granules of enterosorbents at 0-2°C (Vysekantsev I. et al., 2011). The safety of complexes "carrier-cells" was determined by the number of macrocolonies which were formed by cells immobilized on a single granule of a carrier (Martsenyuk V. et al., 2012). **Investigated biological properties**: resistance to gastric juice (Martius F. et al., 2008; Lee K, Heo T., 2000) and to bile salts (Sharaf A.N. et al, 2009; Charteries W. et al, 1998), antibiotics sensibility – Kirby-Bauer disk method (1966), antagonistic activity by two-layer method with MICA measurement (Kulchitskaya M., 2006), saccharolytic activity, restore induction of the gut microbiota in animals with experimental dysbiosis. Animals' experimental dysbiosis was caused by oral administration of ampiox and metronidazole (5 and 2 mg – for mice, 15 and 10 mg – for rats) during three days. Animals' immunosuppression was caused by hydrocortisone acetate subcutaneously. The structure of complexes "carrier-cells" was studied by scanning electron microscopy.

RESULTS





When freezing to -196°C and to -80°C the part of immobilized complexes of *S.boulardii* died. During subsequent storage at these temperatures for 1 year (time of observation) there was no additional death of all three immobilized probiotics.

After immobilization and subsequent storage at low temperatures probiotics remained resistance to threshold concentrations of HCl, antibiotic sensitivity, range and severity of antagonistic activity against pathogenic and opportunistic bacteria and fungi of the genus *Candida*, saccharolytic activity spectra. Probiotic immobilized complex increased the resistance to bile salts.

After therapy with immobilized on enterosorbents probiotics in animals with experimental dysbiosis intestine microbiota significantly faster recovered and reduced the clinical manifestations of digestive function disorders of the gastrointestinal tract. Animals treated with free probiotic cells and mixture of probiotics cells with enterosorbents were a comparison group. In animals that received immobilized *S.boulardii*, it was noted a longer persistence of yeast cells in colon mucin.

In immunosuppressed mice on the background of dysbiosis it was observed a translocation of intestinal microflora in the internal organs. Immobilized probiotics provided faster eradication of intestinal microflora from the internal organs.

Storage at -80°C, -196°C did not affect the therapeutic activity of the immobilized probiotics.

Microphotos (SEM) of free and immobilized on enterosorbents *S.boulardii* before and after freezing to -196°C: a - cells immobilized on Sorbex (control 1); b - cells immobilized on Sorbex, after freezing; c - cells immobilized on the SUMS-1 (control 2); d - cells immobilized on the SUMS-1, after freezing.

CONCLUSIONS

1. Immobilization on enterosorbents and subsequent storage at -80°C and -196°C do not affect the biological properties of probiotics *S.boulardii*, *B.bifidum*, *L.bulgaricus*
2. Immobilized on enterosorbents probiotics exhibit more pronounced therapeutic effect in experimental dysbiosis correction in laboratory animals compared to free cells of probiotics and mixtures of enterosorbents with free cells.
3. The storage at -80°C and -196°C do not affect the therapeutic efficacy of the immobilized probiotics in the experiment.