POSSIBLE APPLICATIONS OF *SACCHAROMYCES BOULARDII* IN SPACE MEDICINE

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During space flight, the astronaut's body is continuously exposed to various stress factors, among which microgravity, cosmic radiation and isolation are the most important [1-3]. It has been established that these factors can have a negative impact on the health and working capacity of the crew members, leading to the development of infectious diseases and dysbiosis, the occurrence of which is due to a change in the composition of the microbiota, an increase in the number of opportunistic microflora and a decrease in the reactivity of the immune system [4]. Therefore, as a preventive measure, it is advisable to use probiotic agents, the reception of which is intended to alleviate the consequences of space flight. For example, lyophilized preparations based on the yeast *Saccharomyces boulardii*, which are widely used in clinical practice for the treatment and prevention of the development of a number of diseases of the gastrointestinal tract. The yeast *S. boulardii* has pronounced probiotic, immunostimulating, anti-inflammatory, antitoxic properties that contribute to the rapid restoration of normal intestinal microbiota and the destruction of pathogenic microorganisms at an early stage [5-7].

The aim of recent work was to study the radiosensitivity and radioprotective properties of probiotic strains of *S. boulardii*. A series of radiobiological experiments was carried out based on Laboratory of Radiation Biology JINR (Dubna).

Materials and Methods

Probiotic strains of the yeast *S. boulardii* isolated from the preparations «Enterol» (Biocodex, France) and «Cosm-o-tentic» (Putramos, Belgium), laboratory yeast strains *Saccharomyces cerevisiae* 711a (*MAT*a *ade2*) and XS800 (*MAT*a/*MATα RAD+/RAD+*) derived from S288C were used. Strains were grown in a rotary shaker (200 rpm) at 30°C or 37°C in YPD medium. Cellular growth was monitored by measure of the optical density (OD) at 600 nm.

Chromosomal DNA was isolated by a miniprep method using glass balls. Amplification of DNA fragments was carried out using T100 ThermalCycler (BioRad).We used the following primers to study*CAN1* gene: CAN-F (5'-TCT-GTC-GTC-AAT-CGA-AAG-3') and CAN-R (5'-TTC-GGT-GTA-TGA-CTT-ATG-AGG-GTG-3'). Primers were synthesized by Syntol (Moscow). Sequencing of amplified fragments (~200-600 n) was performed by Syntol (Moscow). The analysis of the DNA nucleotide sequence of selected mutants was performed using paket CodonCode Aligner and BLAST. Referenced strain was S288C.

Result and Discussion

Genetic characterization of S. boulardii. Our study found that *S. boulardii* is a prototroph, because it grows on selective YNB mediums without the addition of amino acids. Cells are respiratory competence because they grew on media with glycerol, non-fermentable sugar. It does not sporulate due to the presence of a mutation in the *MAT* loci, which is critical for sporulation [8].

Since the preparation will be taken by astronauts, it was advisable to study the growth of probiotic strains at human body temperature (37°C). It was found that there is no significant difference between the growth of strains *S. boulardii* and *S. cerevisiae* at both temperatures (Figure 1).

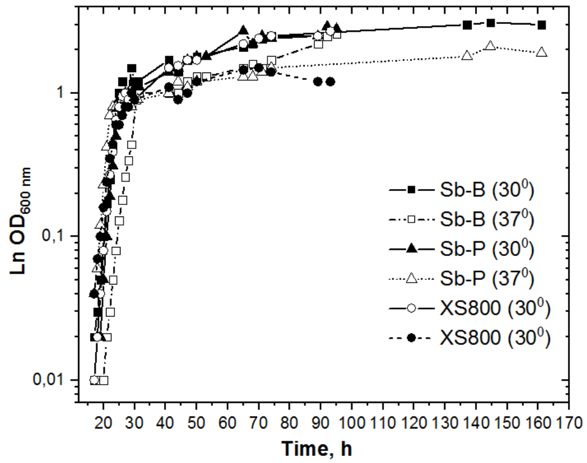

Figure 1. Growth curves in YPD at 30°C or 37°C of *S. boulardii* (Sb-B, Sb-P) and *S. cerevisiae* (XS800)

Radiobiological characteristics of S. boulardii. When studying the radiosensitivity to the action of proton irradiation (150 MeV, 0.54 keV/mkm), it was found that with an increase in the irradiation dose, the survival of haploid *S. cerevisiae* yeast cells 711a decreases (Figure2). At the same time, the survival of two probiotic *S. boulardii* strains and one diploid laboratory *S. cerevisiae* strain XS800 did not practically change in the used dose range.

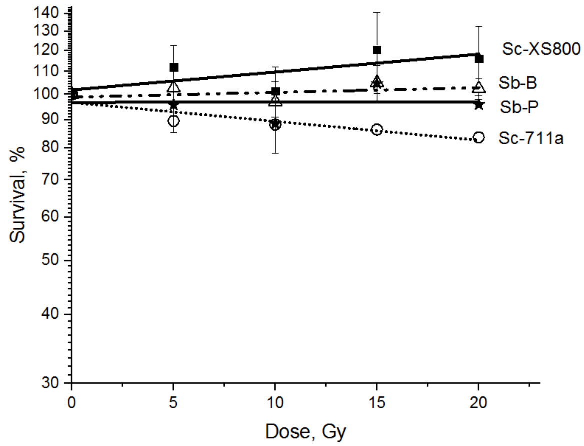

Figure 2. Survival of bidding yeast cells of *S. cerevisiae* and *S. boulardii* after proton irradiation (150 MeV, 0.54 keV/mkm)

To study mutability one often uses drug-resistance assay in particular mutations in single *CAN1* gene determining resistance to canavanine.

Unfortunately *S. boulardii* cells were found to be canavanine-resistant. Analysis of the nucleotide sequences showed that both *S. boulardii* strains differ from *S. cerevisiae* and among themselves. Their genotypes contain two different mutations in the *CAN1* gene compared to the reference strain *S. cerevisiae*: Sb-B (Biocodex) and Sb-ANCC MYA-796 (SGB) – [C1445G A1600G], Sb-P (Putramos) – [T526G A1600G]. Therefore, we used 5-fluorocytosine (5-FC) and alpha-aminoadipate (AA) to test for mutability.

In the study of drug resistance, a linear relationship was found for the haploid strain *S. cerevisiae* 711a: with an increase in the radiation dose, the frequency of induction of resistance mutations to 5-FC and AA increases (Figure3). For the *S.boulardii* strain Sb-B and diploid *S. cerevisiae* strain, we did not show mutation induction, may be due to their diploidy.

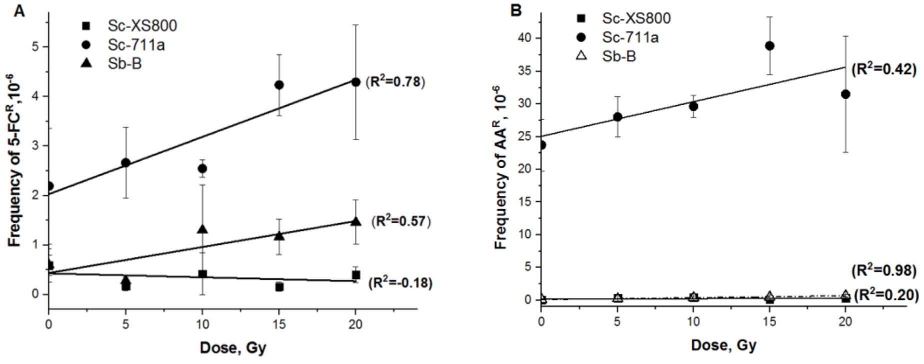

Figure 3. Spontaneous frequency of resistance to 5-flucitozine (A) and α- aminoadipic acid (B)

Conclusion

In results we show normal growth at 37°C, radioresistance and low mutation level of probiotic strains. Preparations of *S. boulardii* are perspective for solution of health problems of astronauts and for study their probiotic and radioprotective properties during space flight.

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