
# Effects of proton irradiation on probiotic strains

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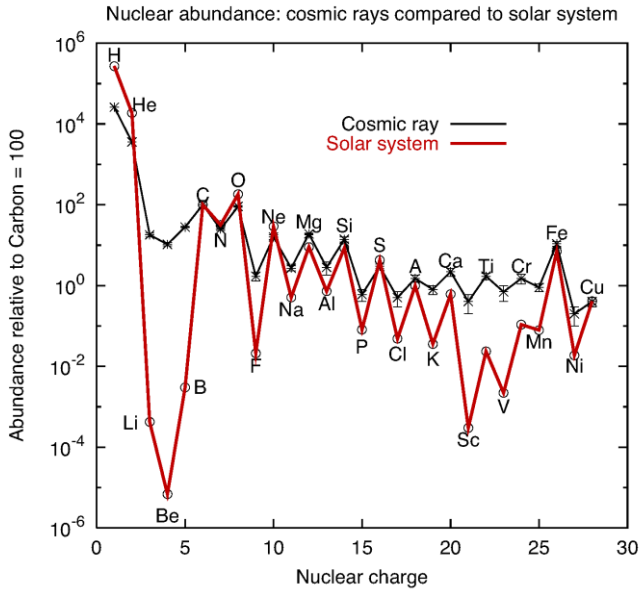
The actuality and importance of investigations of proton and other ions action were caused by using in medicine and problem of cosmic radiation protection. Cosmic radiation is comprised from energetic protons (~95%) and heavy nuclei (Li, B, C, N, O, etc.) with energy of >10 MeV/u. Since probiotics use at space stations, to study evolution and changes in the properties of probiotics under stressful flight conditions, including proton irradiation is need. Here we report the important radiobiological characteristics of two yeast probiotic agents – *Biocodex* (France) and *Puratos* (Belgium) after proton irradiation.



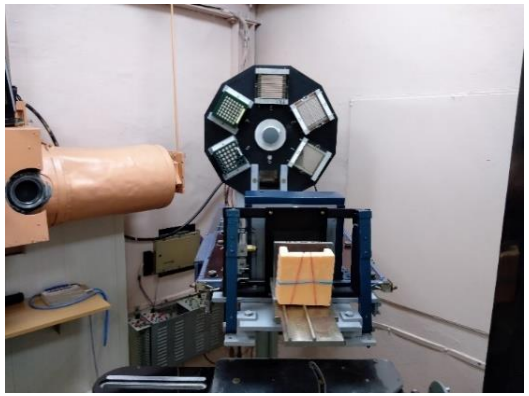

**Equipment**:

In laboratory conditions, the source of protons was the phasotron.

The phasotron has 10 beam channels employed for experiments with pi-mesons, muons, neutrons and protons. Five secondary beams are intended for medical research, mainly in oncotherapy. Proton exposure was performed at the clinical proton beam facility of the Medico-Technical Complex (LNP JINR, Dubna). Lyophilized cells in Eppendorf tubes were irradiated with unmodified **170 MeV proton beams (LET 0.54 keV/μm).** Dose rate was **0.6 Gy/min**. A 52-mm Plexiglas absorber was used. Calibration was performed by ion chamber TM30013 (PTM UNIDOS-E).

Phasotron

Operating console



Secondary beam with irradiated samples

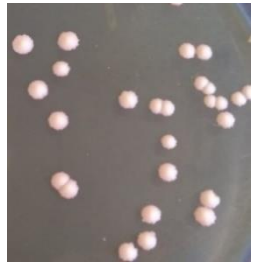
**Strains:**

Yeast *Saccharomyces*, liophylized clonal cultures:

*Saccharomyces cerevisiae* (derived from laboratory X2180-1A strain) **D71**

*Saccharomyces boulardii* (“Enterol”, *Biocodex*, France) **Sb-B**

*Saccharomyces boulardii* (“Cosm-O-tentic”, *Puratos*, Belgium) **Sb-P**





*Genotypes of homozygotes:*

*diploid D71 GLU SUC gal mal FRU ade1* [Ery221$^{R}$ Chl321$^{R}$ Oli7$^{R}$]

*diploid Sb-B GLU SUC gal MAL FRU can1 -C1445G, A1600G* [Ery$^{R}$]

*tetraploid Sb-P GLU SUC gal MAL FRU can1 -T526G, A1600G*

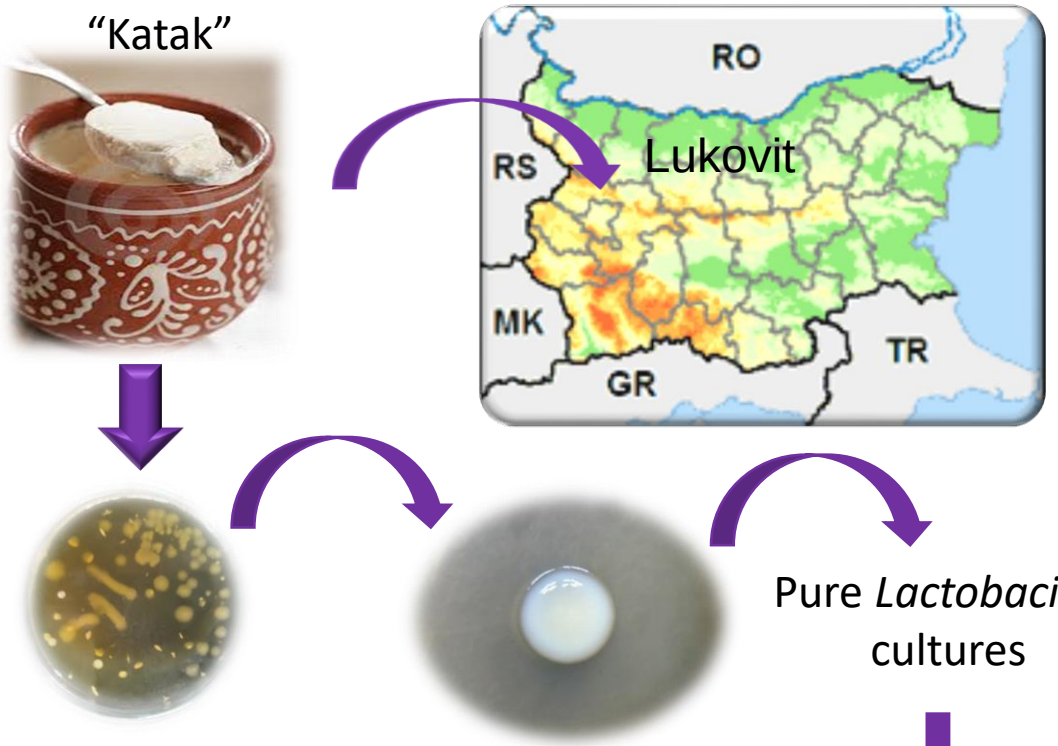
The results of the previous study strongly indicate a close relatedness of *Saccharomyces boulardii,* derived from the *Biocodex* (France) and *Puratos* (Belgium) probiotic agents, to *Saccharomyces cerevisiae* laboratory strains. Morphological and physiological characteristics of commercial *S. boulardii* were consistent with those of laboratory strain of *S. cerevisiae.* However, *S. boulardii* isolates show some differences, particularly in relation to nucleotide sequences, ploidy, antibiotic resistance, which are important characteristics for a microorganism to be used as a probiotics.

Lyophilized preparations of the yeast *Saccharomyces boulardii* have been used for the treatment of antibiotic-induced gastrointestinal disorders and acute enteritis. Also probiotics have the potential to improve stress conditions that manifest during space travel, such as gastrointestinal disorders, dermatitis, and respiratory infections.

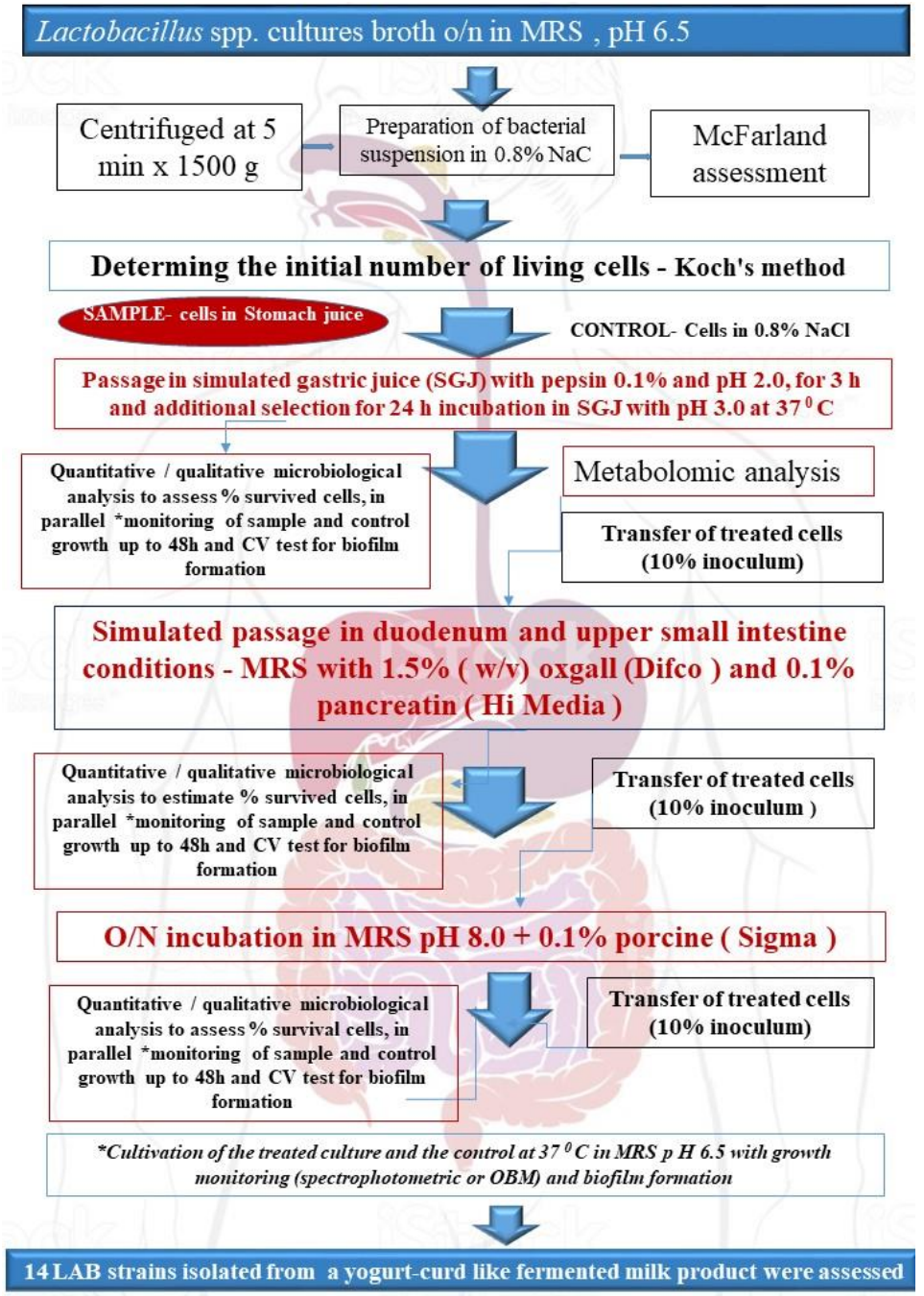
Lyophilizes of used strains were obtained from pure cloned cultures.
Viability lyophilized cultures ~10%

Tabletop freeze drying FreeZone with cooling system -50°C, 2.5 liters, Labconco (Moscow)

# Selected candidate-probiotic Lactic acid bacteria (LAB)

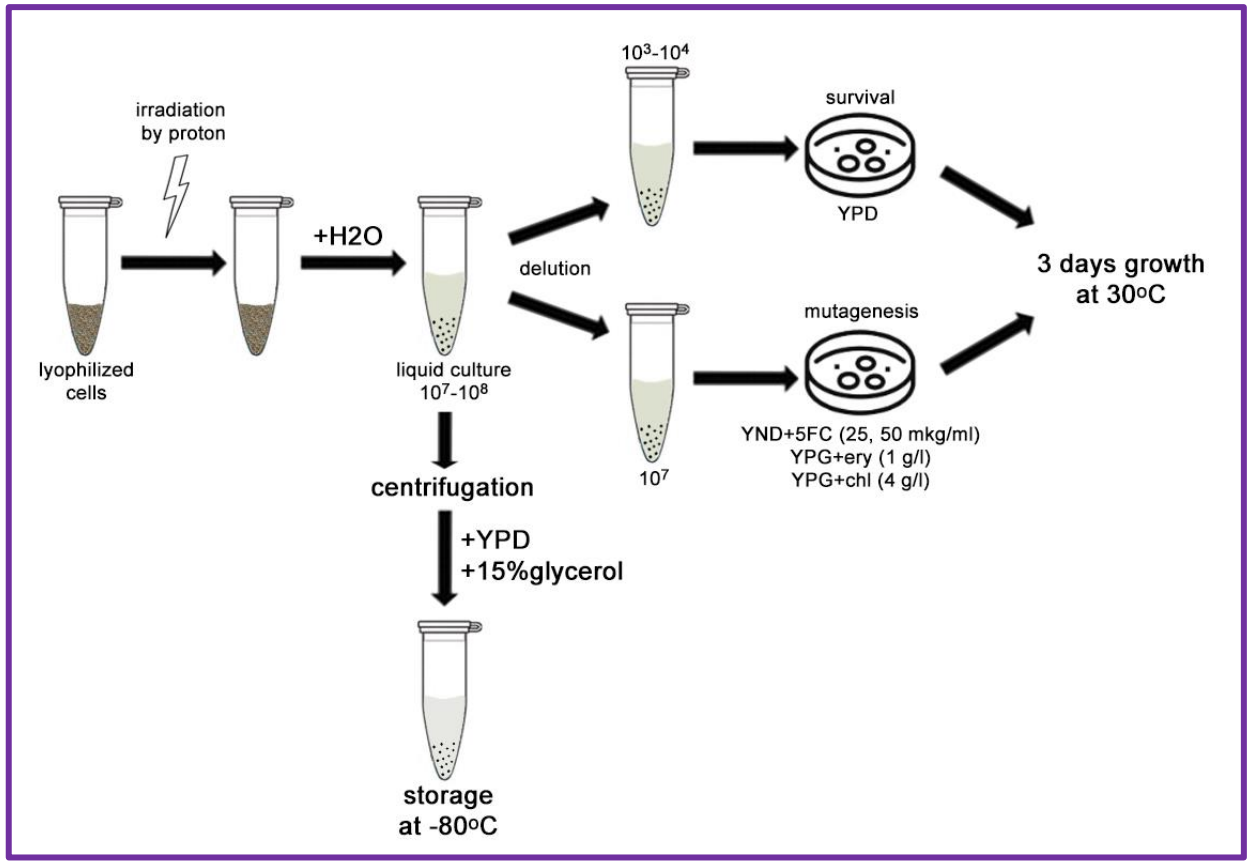



Two strains were selected, from the autochthonous microbiota of home-made sample of "Katak" from the town of Lukovit, identified **as *Lactiplantibacillus plantarum L6 and L9*** *(*Dobreva L. 2023, PhD thesis). Strain MIX10 - mixture of several strains.

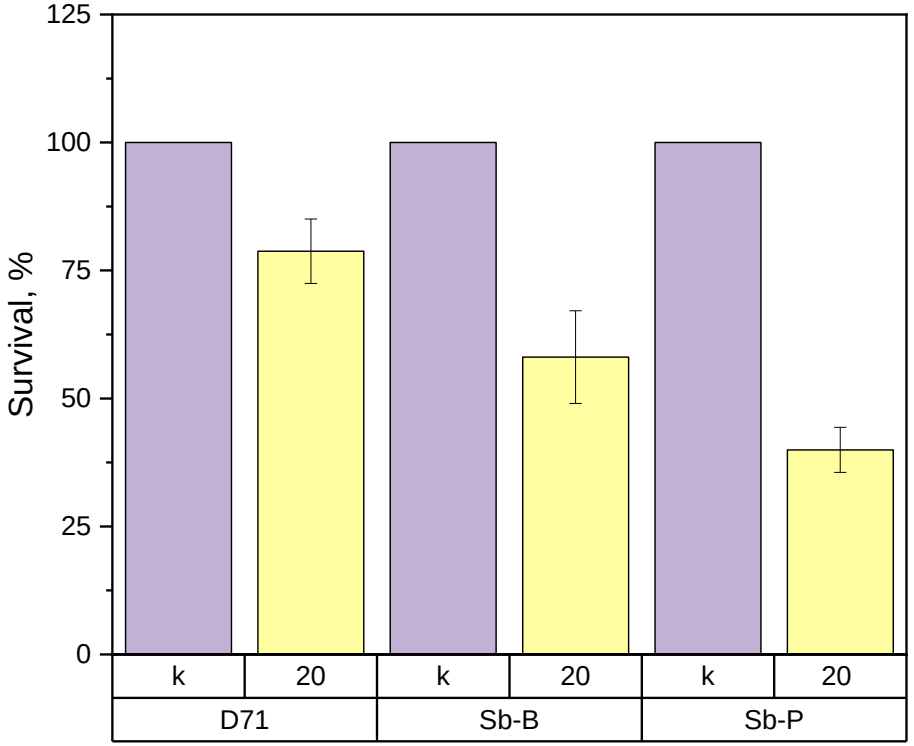


# Proton irradiation (150 MeV, 0.54 keV/mkm)

## Experimental design for yeast probiotics

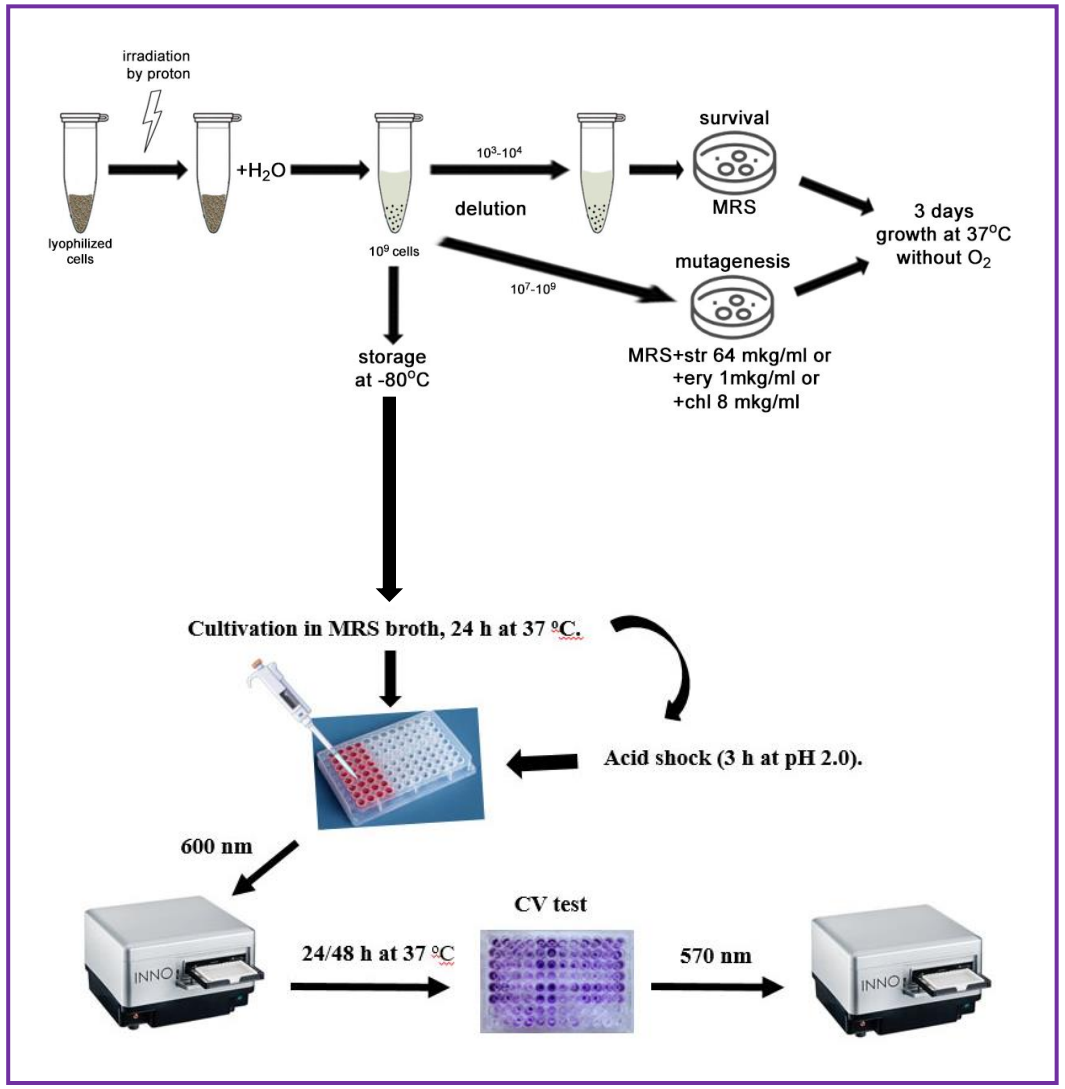



## Survival

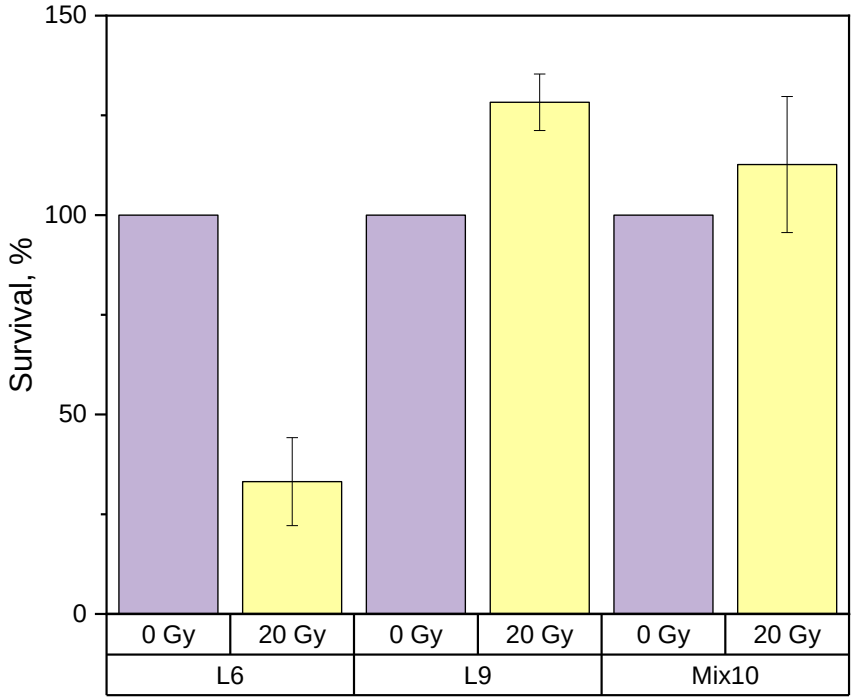



Commercial probiotic strains are more sensitive than the laboratory strain.
The highest radiosensitivity was observed for the tetraploid strain Sb-P.

# Experimental design for bacterial probiotics

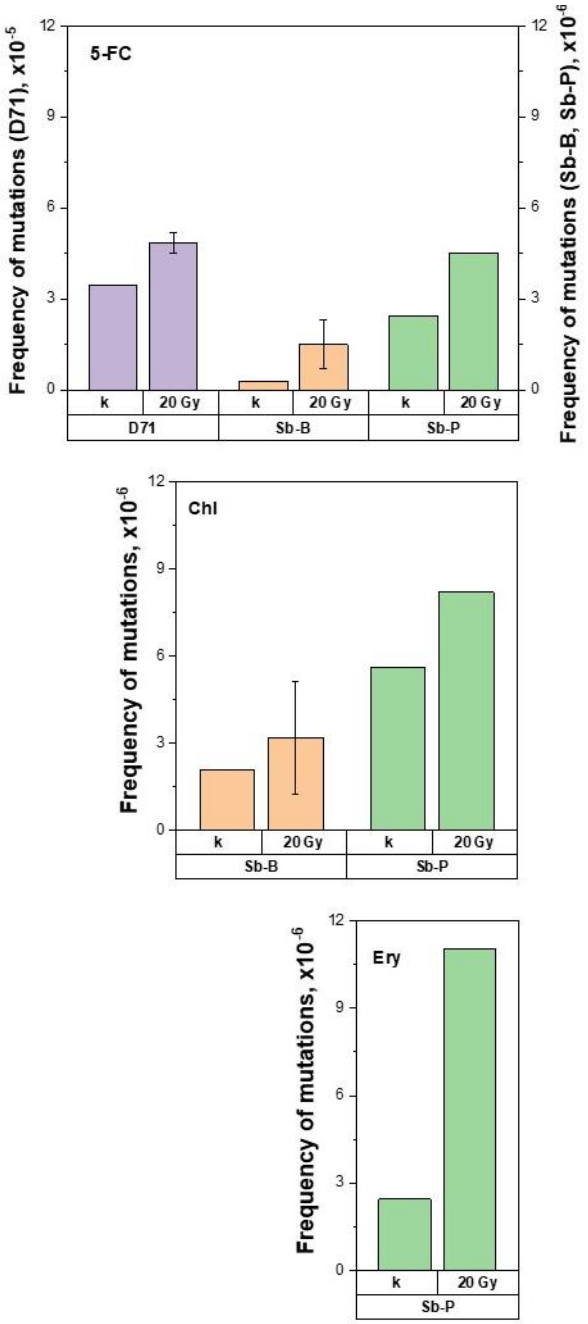



# Survival



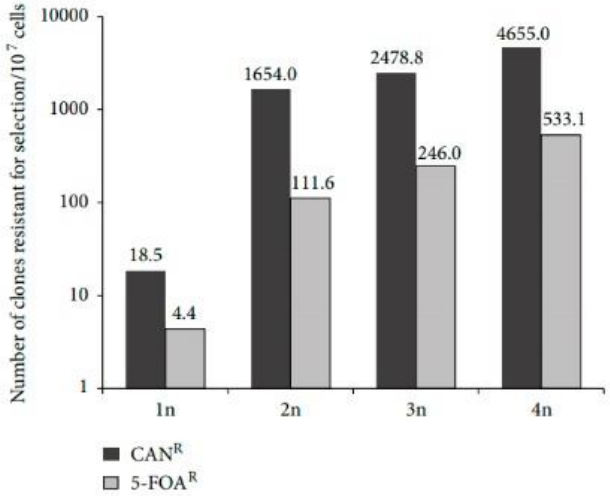

Lactobacillus strains L6 was more sensitive then L9 and Mix10 to proton irradiation.

# Spontaneous and p$^{+}$-induced mutagenesis for yeast strains




It was shown that radiosensitivity and mutability (mutation + homozigotization) of the lyophilized yeast strains were different and corresponded by their ploidy, i.e. *Biocodex* was diploid and *Puratos* was tetraploid. The last of them was more sensitive and probiotic strains were more sensitive than laboratory strain. The frequency of drug$^{R}$-mutations was respectively low.

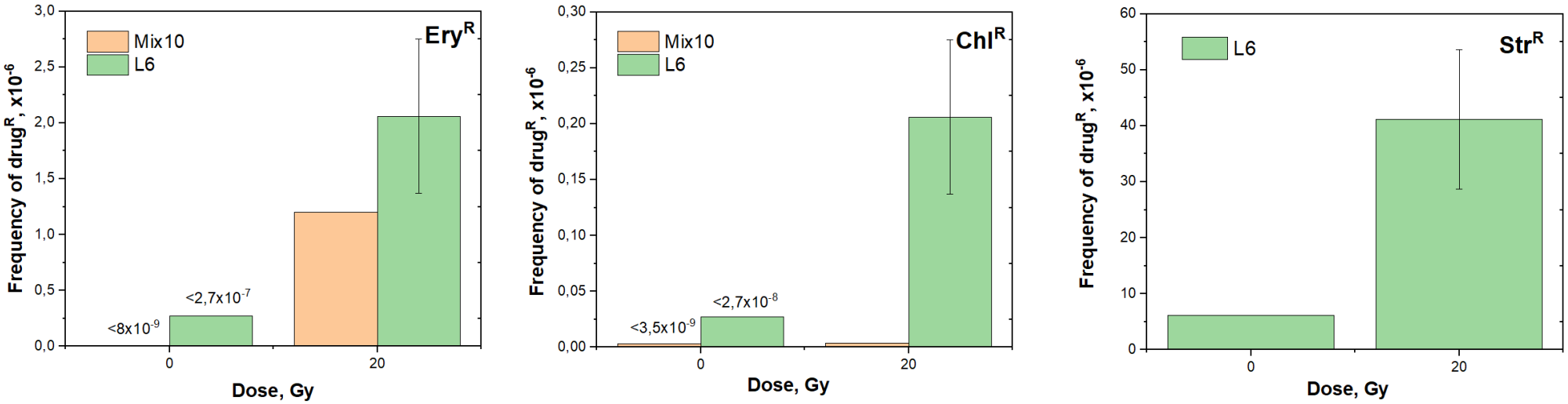
The spontaneous mutagenesis level was measured in strains from 1n to 4n in the BY474X genetic background. All strains have only one functional copy of gene *CAN1* and *URA3*, which serves as the mutagenesis marker locus allows tracking of the frequency of marker loss. Almost two orders of magnitude in mutation frequency between haploid and diploid strains [*Alabrudzinska et al., 2011* ], an additional increase in mutation frequency with the rising ploidy was detected. However, this increase was not as spectacular as between haploid and diploid strains; the mutation levels doubled with each additional genome copy.




*M. Alabrudzinska, M. Skoneczny, and A. Skoneczna, "Dipoid-specific genome stability genes of S. cerevisiae: genomic screen reveals haploidization as an escape from persisting DNA rearrangement stress", PLoS One, 2011, vol. 6, no. 6, article e21124.*

# Spontaneous and $p^+$-induced mutagenesis for bacterial probiotics

Drug$^R$-mutations for lactobacillus strains L6 induced more efficiently. The growth of both lyophilized lactobacillus strains in MRS broth and in modified MRS with a single carbon source FOS (Actilight® Fibre - Actilight®P950 (Picco, LTD)) was not affected by proton irradiation. Moreover, L9 strain showed enhanced growth. While the viability in the presence of 10% v/v pancreatin was lower for the both irradiated with 20 Gy strains. Further characterization of the genetic basis of resistance to stress conditions will allow the selection of probiotics for the purpose of long-duration space missions with non-harmful exposures to ionizing radiation.

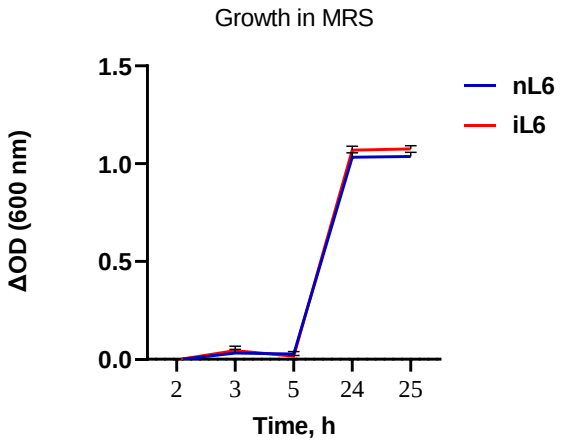


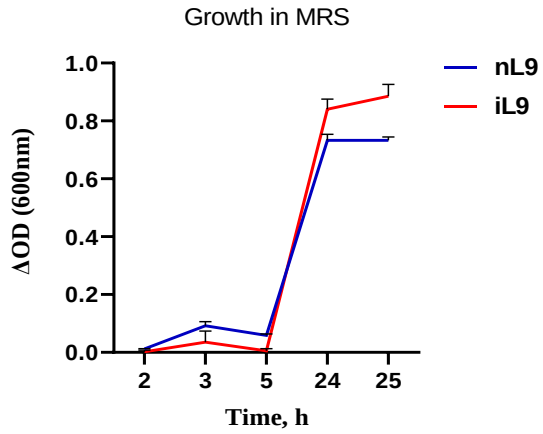

Spontaneous frequency of drug$^R$ mutations were very small <$10^{-8}$, and induced efficiently in L6 but not Mix10.

**Str** has a bactericidal effect by binding to the 30S subunit of the bacterial ribosome, which subsequently leads to the inhibition of protein synthesis.
**Ery** and **Chl** disrupt protein synthesis on ribosomes, reversibly bind to 23S rRNA of the 50S subunit of bacterial ribosomes and suppress the translocation of the synthesized peptidyl-tRNA molecule from the acceptor site of the ribosome to the donor site. The binding sites for macrolides and chloramphenicol on the 50S subunit of the ribosome are located nearby. Antibiotic resistance can arise due to mutations in ribosomal RNA or in some protein ribosomal factor. As a result, the properties of this ligand change. In this case, the inhibitor, that is, the antibiotic that binds in this or an adjacent place, simply ceases to have an effect.

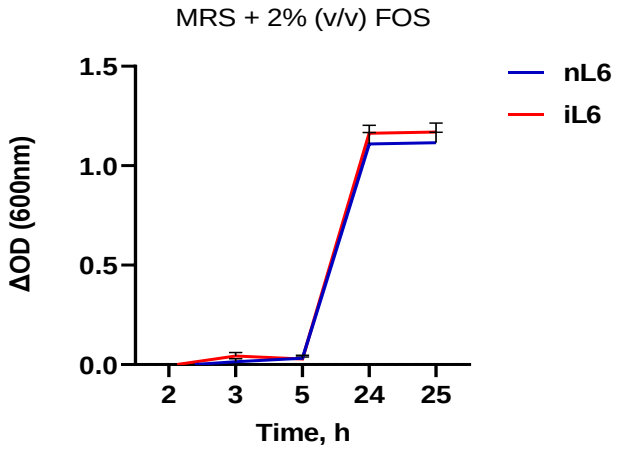
# Growth assessment of the probiotic strains

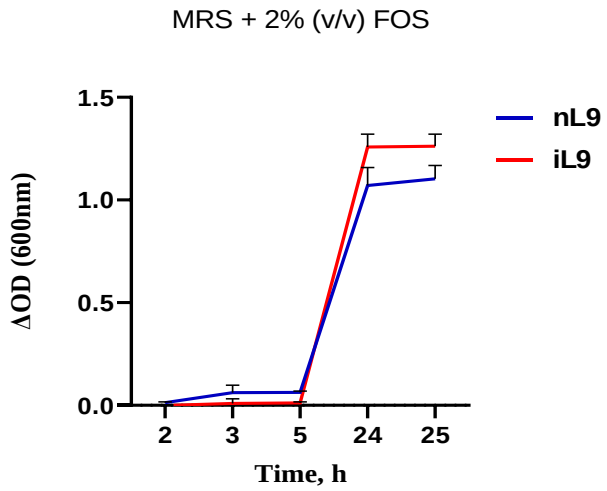
## Irradiated strains with p+ (iL6 and iL9 ) and controls - non irradiated (nL6 and nL9)



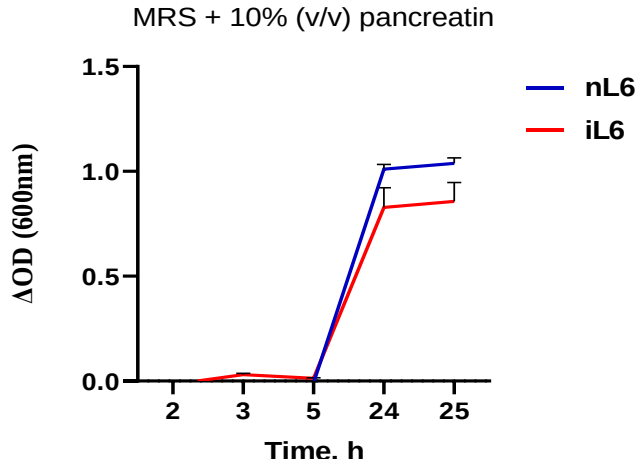



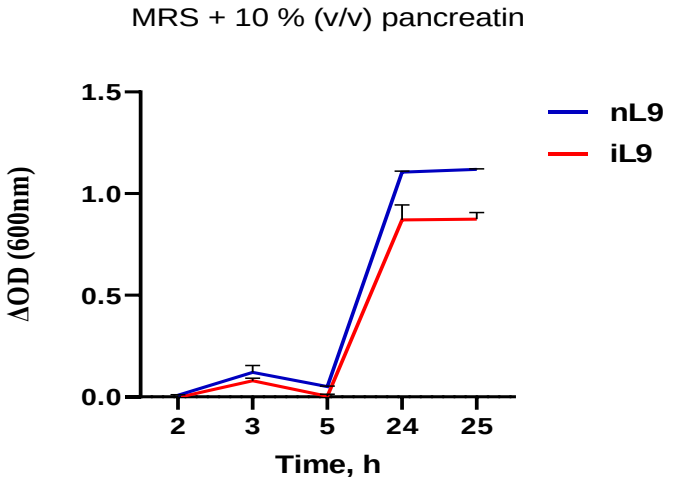

Probiotic *L. plantarum* strains in MRS broth (HiMedia, India)




Probiotic *L. plantarum* strains in modified MRS broth (HiMedia, India) supplemented with FOS (2% w/v) as sole carbon source



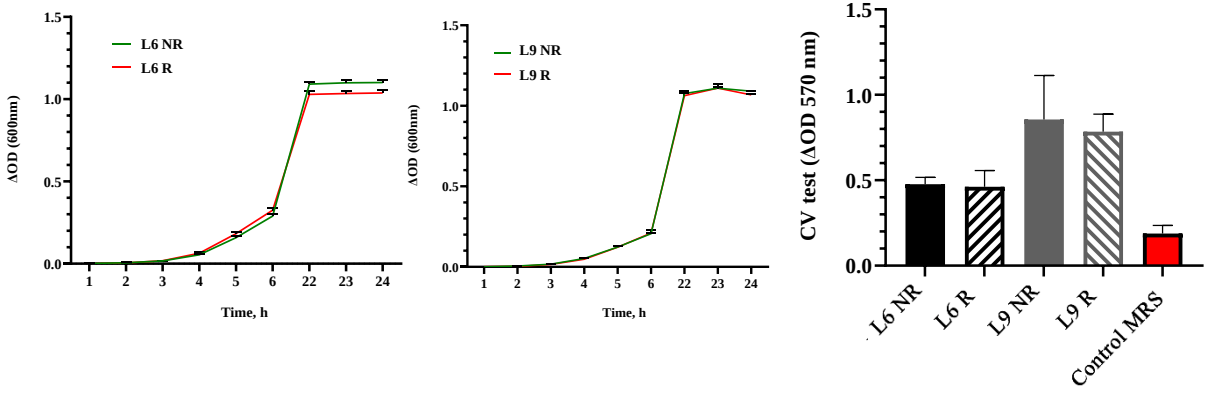

Probiotic *L. plantarum* strains in modified MRS broth (HiMedia, India) supplemented with pancreatin (10% w/v) to simulate a passage upper part of the gut

## Effect of irradiation on technologically relevant characteristics of LAB

Technologically relevant characteristics such as proteolytic activity and milk coagulation were not significantly affected. Therefore, after additional characterization the strains can be implemented in food and as probiotics/ supplements in space missions

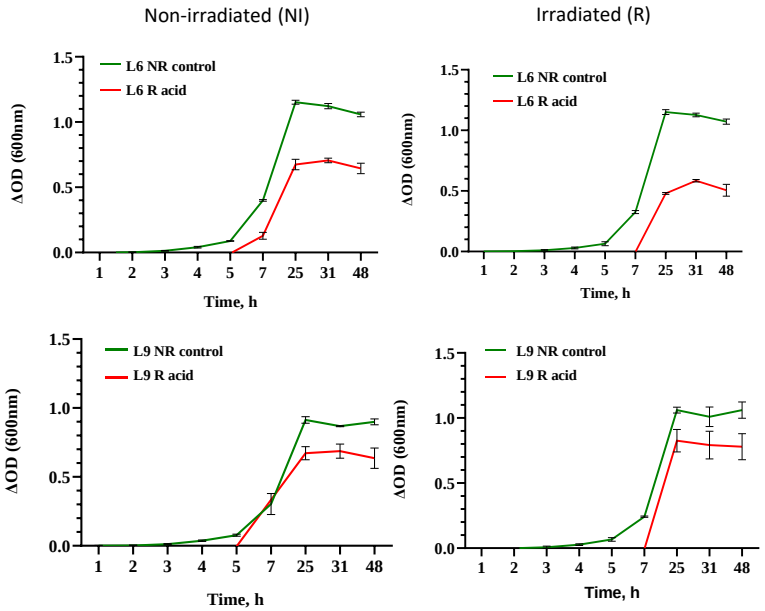
| Sample | Zone, mm |
|---|---|
| L6 NR control | 19 |
| L6 NR acid | 17 |
| L6 R control | 19 |
| L6 R acid | 17 |
| L9 NR control | 24 |
| L9 NR acid | 20 |
| L9 R control | 25 |
| L9 R acid | 22 |

## Growth and biofilm formation of LAB after irradiation

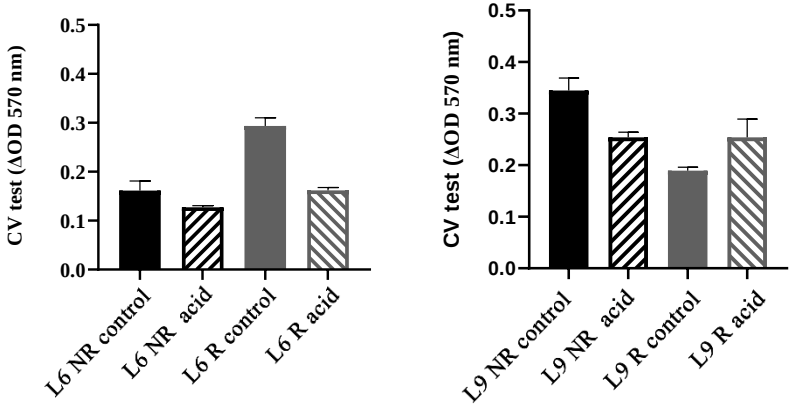



Spectrophotometric monitoring of growth and biofilm formation of L. plantarum L6 and L9 strains after irradiation with a dose of 20 Gy $P^+$ showed high radiation resistance. No statistically significant difference was observed between the control (non-irradiated) and the samples biofilms.

## *In vitro* resistance to acid shock




Samples shown strain-specific viability after acid shock. No significant change in acid resistance of strains before and after irradiation with proton beams was observed. Similar results were obtained for biofilm formation.



## Results:

Irradiation with protons at a dose of 20 Gy showed that ionizing radiation has different effects on the used probiotic strains.

- ✓ Radiosensitivity and mutability of the lyophilized yeast strains were different and corresponded by their ploidy - *Biocodex* was diploid and *Puratos* was tetraploid. The last of them was more sensitive and probiotic strains were more sensitive than laboratory strain. Moreover, *Puratos* proved to be more mutable.

- ✓ Lactobacillus strains L6 was more sensitive then L9 and Mix10 to proton irradiation. $Drug^R$-mutations for strain L6 induced more efficiently. The growth of both lyophilized lactobacillus strains L6 and L9 in MRS broth and in modified MRS with a single carbon source FOS was not affected by proton irradiation and L9 strain also showed enhanced growth.

- ✓ Radiation had no effect on growth rate and formation of biofilms of L6 and L9 strains. Also no significant change in acid resistance of strains before and after irradiation with proton beams was observed.

## Conclusion:

The obtained data allow us to choose between several probiotic strains. The probiotic agent “Enterol” (Biocodex, France) and the probiotic lactobacillus strain L9 are more suitable for use in space flights.

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