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## INTRODUCTION

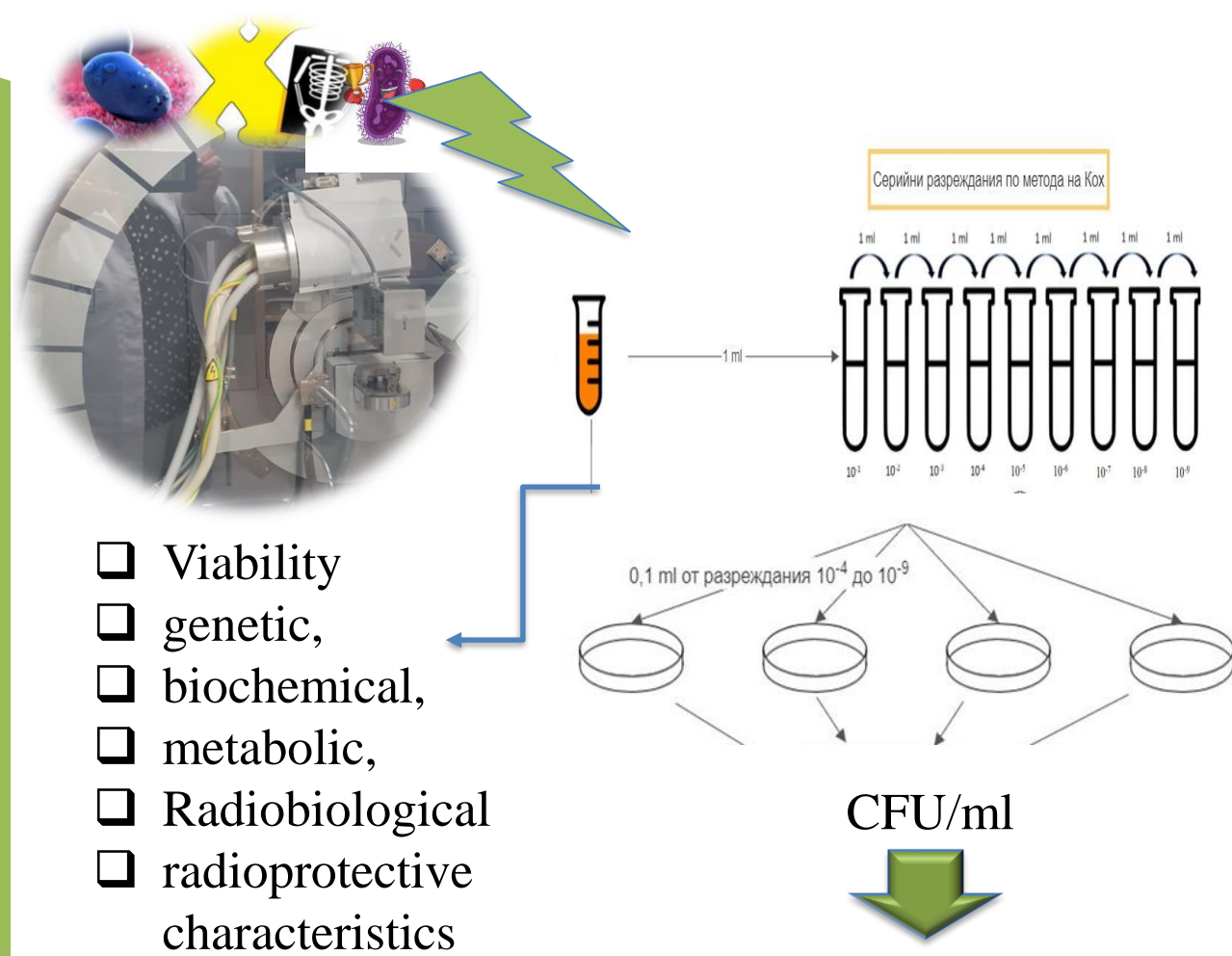
**Probiotic (PRO-FOR AND BIO-LIFE)** are living organisms with beneficial effects on human health. Some of them find application in treatment of diseases, including radiotherapy of cancer. Ground-based research has shown that probiotics have the potential to prevent adverse changes in the microbiome of people with prolonged exposure to ionizing radiation. The irradiation leads microbial inactivation through different direct or indirect effects. The susceptibility of microbes to irradiation, however, differs greatly.

## AIM

In present work we assessed the radiosensitivity of scientifically proven candidate- probiotic lactic acid bacteria (LAB) and commercial yeast. We joint efforts to analyze genetic, biochemical, metabolic, radiobiological and radioprotective characteristics of different probiotic cultures. With this aim two *Saccharomyces* yeast, and original dairy lactobacilli (combined as probiotic multibacterial formula\*) were pre-selected.

## Materials and Methods

Evaluation viability before and after X ray irradiation



- GROWTH and BIOFILMS determination (CV tests) of irradiated LAB strains (S=SAMPLES) v/s controls (non exposed microbial cultures (C=CONTROLS)).
- COAGULATION ACTIVITY in whole milk of irradiated LAB strains
- VIABILITY in laboratory model of undesirable environment
- In vitro capacity of LAB postmetabolites as radioprotective agents.

### LAB STRAINS

Different LAB cultures, isolated from Bulgarian dairy fermented products, made without industrial starters (From the collection of Laboratory "Probiotics & LAB - The Stephan Angeloff Institute of Microbiology, Sofia, Bulgaria.

In vitro strains were assessed as promising probiotics, according to the EFSA's and WHO criteria. Two heterofermentative strains *Lactiplantibacillus plantarum* LS6 and *Lactobacillus* sp. LS2 with a combination between and other lactobacilli were assessed under X- rays exposure (150 kV, 5 mA, doses up to 100 Gy) .

### YEAST STRAINS

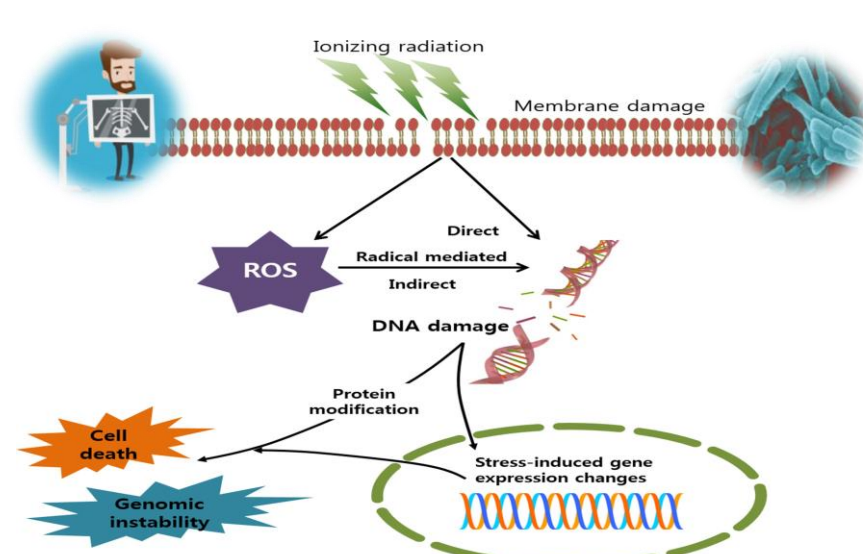
*Saccharomyces boulardii*

*Sb-B*

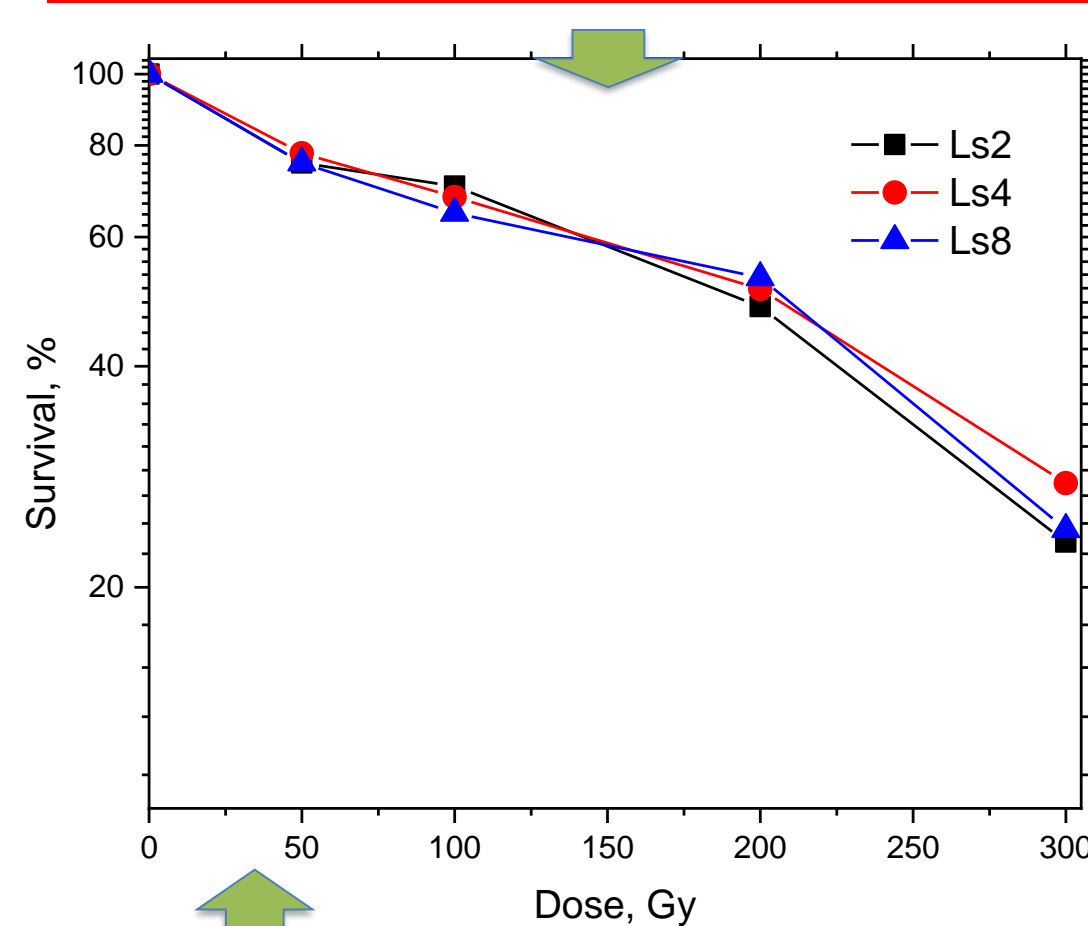
*Saccharomyces cerevisiae* 711a (MATa ade2)

## RESULTS and DISCUSSION

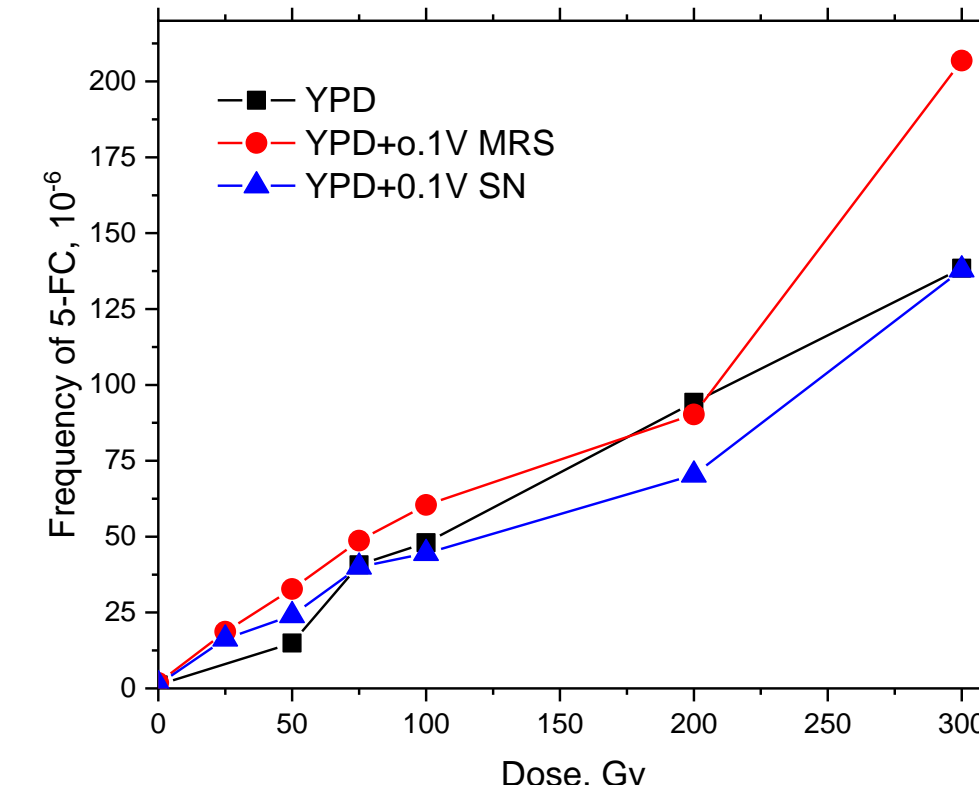
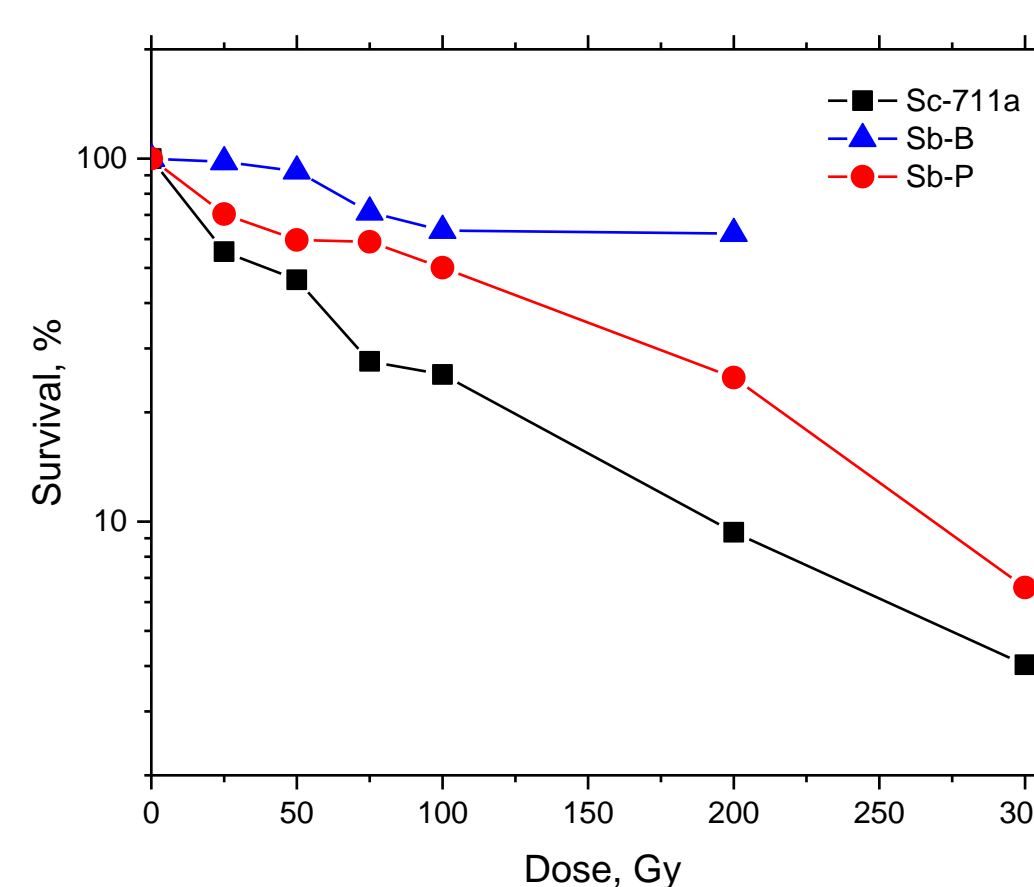
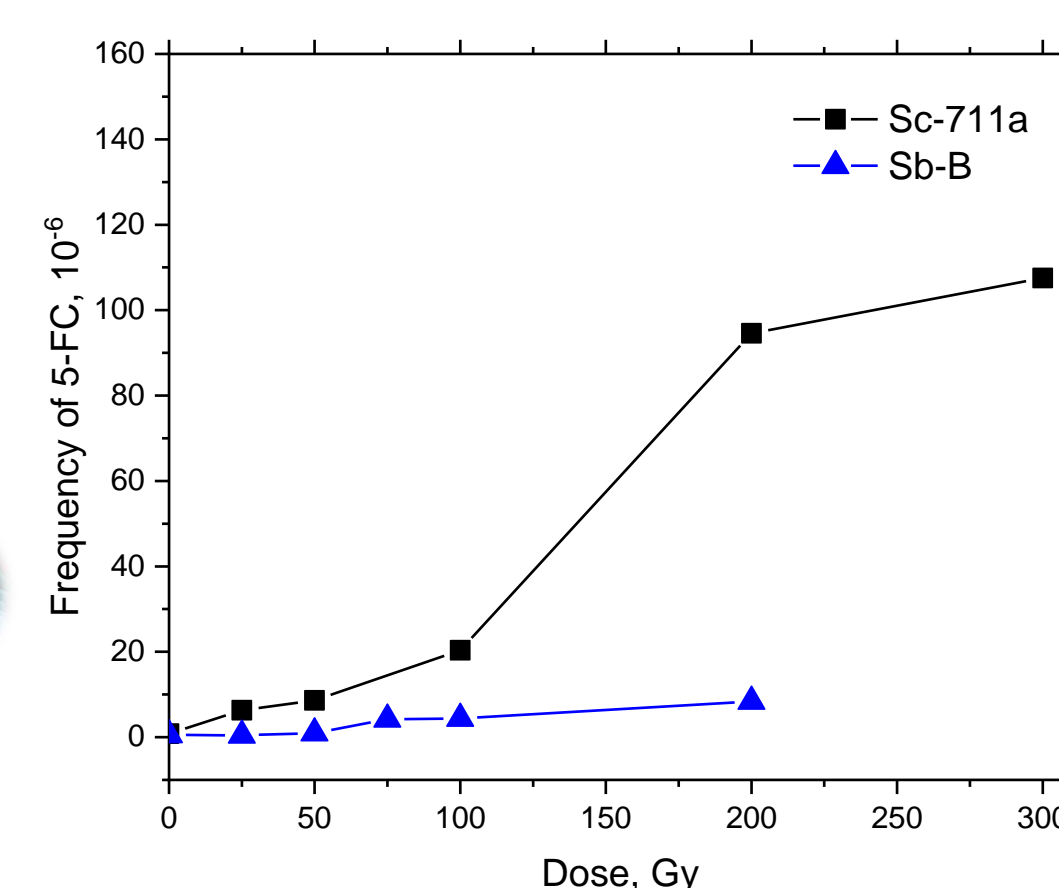
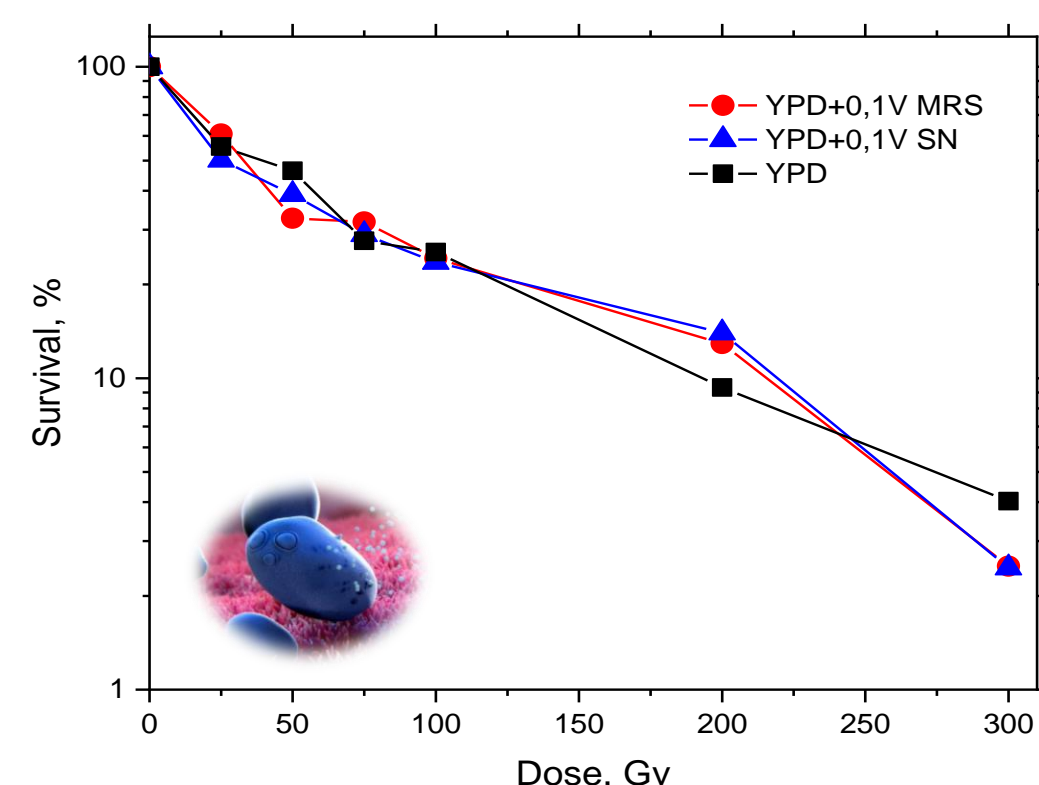
### RADIOSENSITIVITY AND MUTABILITY



LAB Strains S.c. – Ls2, LS4, and probiotic mix Ls8 ( live and lyophilized)  
Source of irradiation – X-rays



Lactobacillus cells are more resistant then yeast cells.



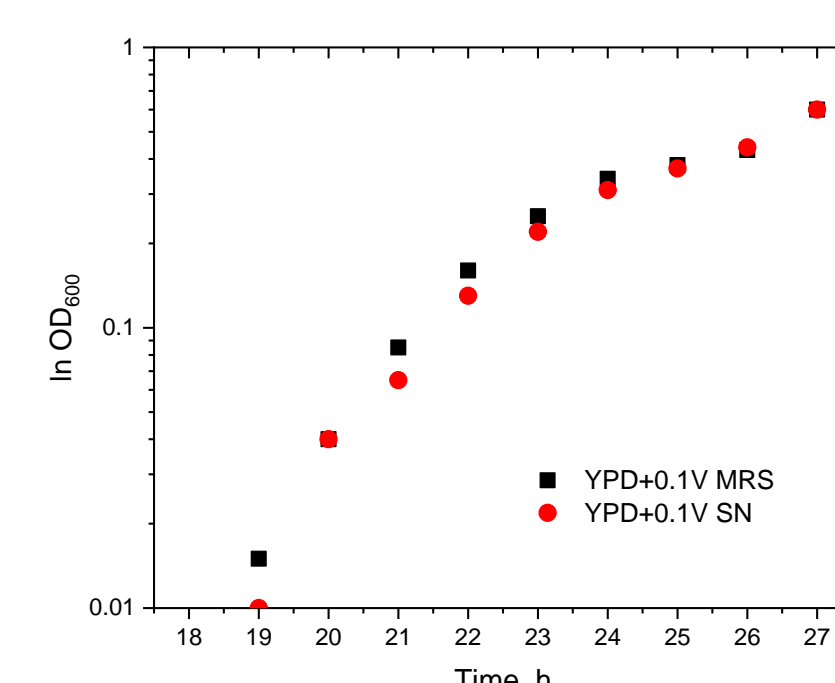
Strain S.c. - 711 a  
Source of irradiation – X-rays

An analysis of the sensitivity of yeast probiotics to hard X-rays (150 kV, 5 mA, doses up to 100 Gy) has been carried out.

The mutability of the strains was assessed by the frequency of 5-fluorocytosine resistance mutation induction.

The probiotic strains differed from each other and had lower frequency of gene mutations then laboratory strains of *Saccharomyces cerevisiae*.

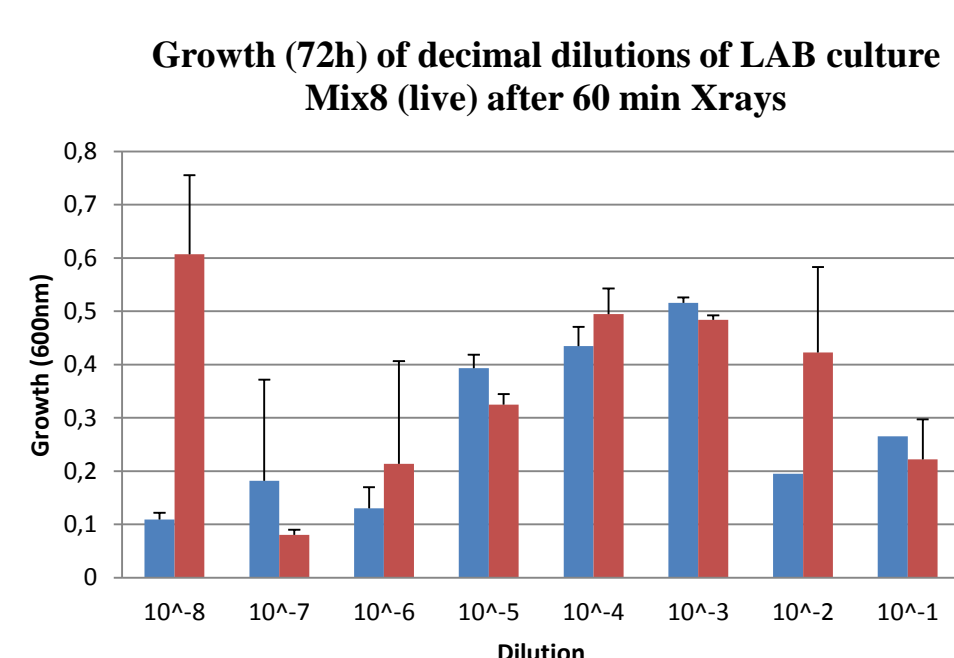
Co-cultivation of yeast *Saccharomyces cerevisiae* with lactobacillus cells-free supernatants (CFS).



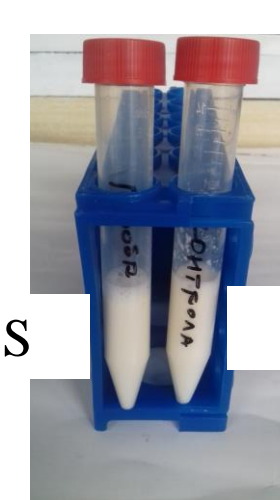
Co-cultivation yeast cells with bacterial media MRS or bacterial supernatant not effect yeast growth

### Co-cultivation of yeast cells with bacterial supernatant not effect sensitivity and mutability of yeast cells.

Live LAB probiotic culture Mix8 in MRS broth



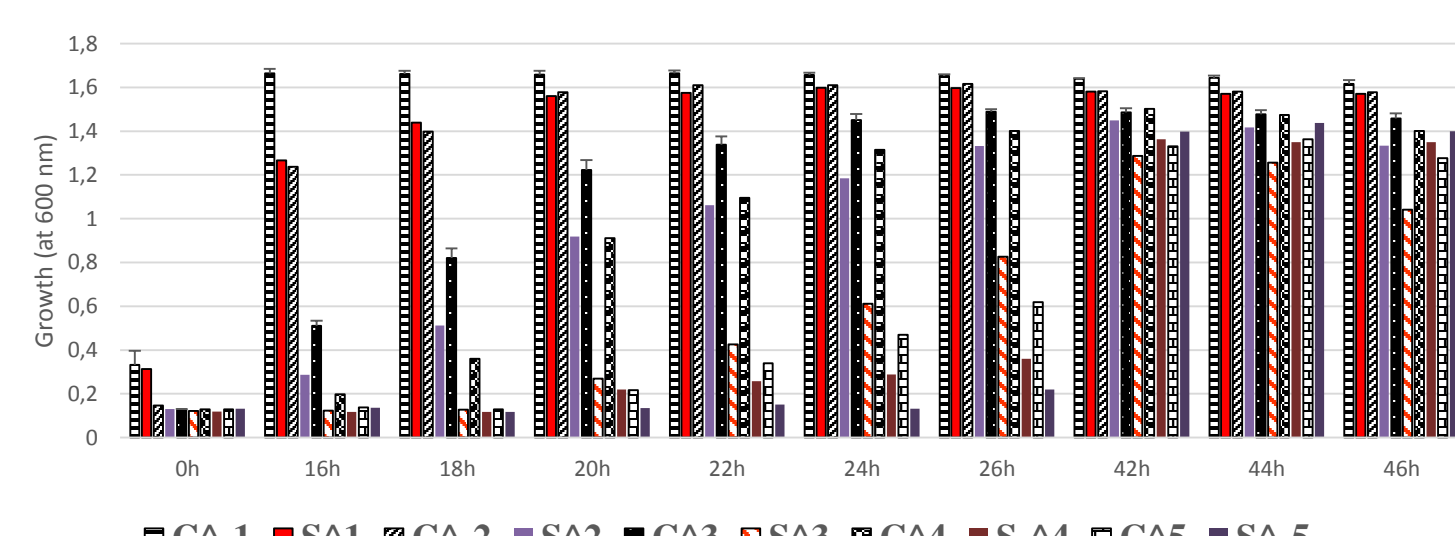
GROWTH of LAB



Milk coagulation activity -preserved

Lyophilized probiotic culture Mix8

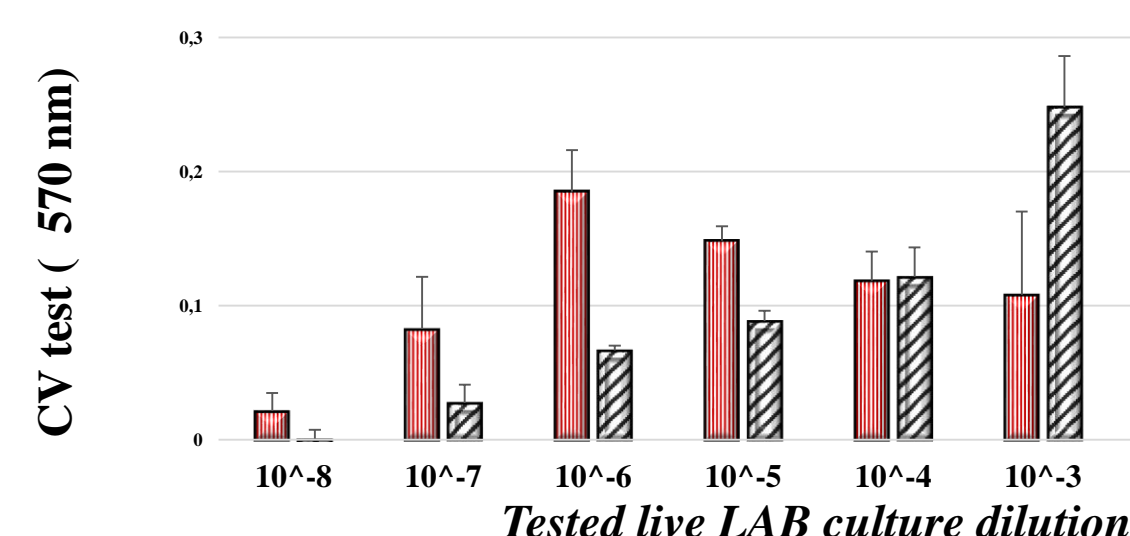
Growth of decimal dilutions of X ray exposed lyophilized LAB culture Mix 8 (S=samples and C- control, non radiated)



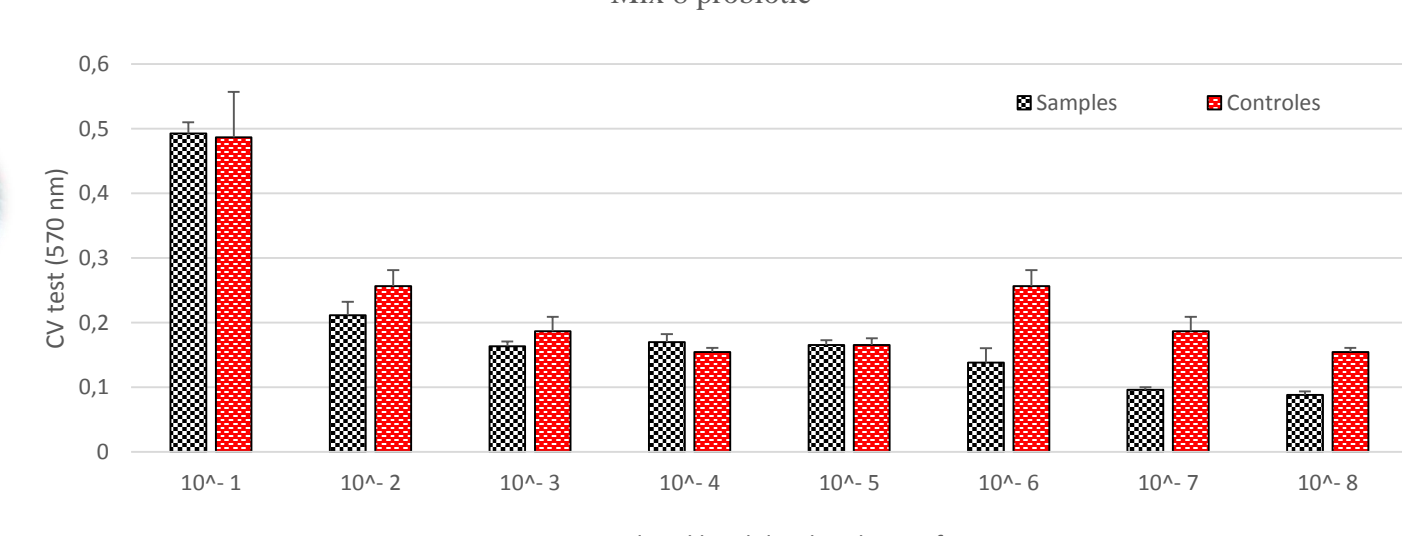
No statistically significant difference of CFU/ml LAB cells under X rays exposure -24 Gray dose.

Lyophilized LAB mix are more sensitive to X rays exposure, than the alive culture .  
The viability of both type of physiological status probiotic Mix8 cultures is probably under quorum sensing dependent control .

### A) LAB biofilms - 60 min X-rays exposure

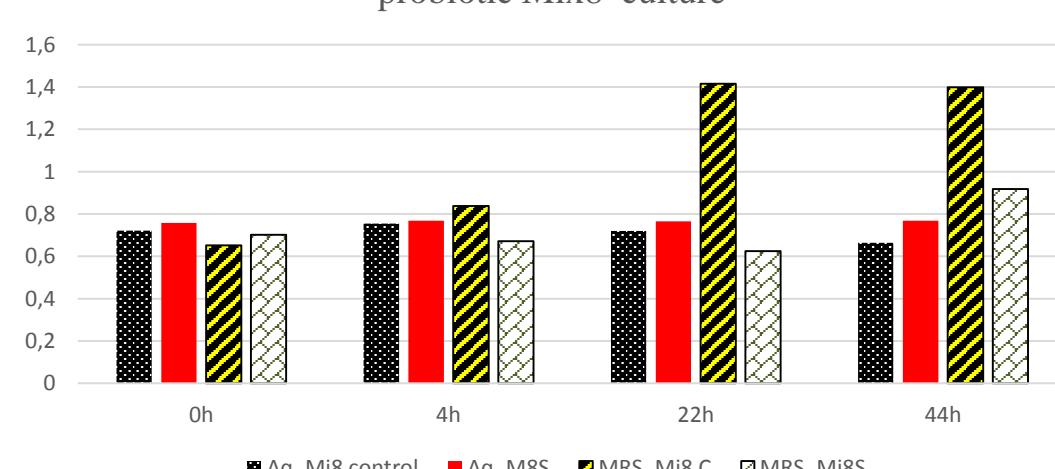


Biofilm formed by cultures derived from decimal dilutions of the irradiated Lyophilized Mix 8 probiotic



Viability of probiotic mix 8 culture in laboratory model of the environment for aquaculture with water from fish farming . The assay was carried out after 30 days storage of irradiated culture and the control at 4oC.

Viability at 37°C in undesirable environments v/s optimal growth in MRS broth (HiMedia, pH 6.5) of probiotic Mix8 culture



## CONCLUSION:

Functional characteristics of probiotic LAB and yeasts are strain-specific and perspective for further characterization under ionizing radiation. In the light of medical application, as well as in space medicine for solution of health problems of astronauts, a larger screening is planned. In the future, new research for other beneficial effects or radio-protective capacity of different postmetabolites/parabiotics of combined yeast-LAB new formulas have to be completed.

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