Compare of proton and X-rays irradiation effects on laboratory, probiotic and bakers's yeast strains

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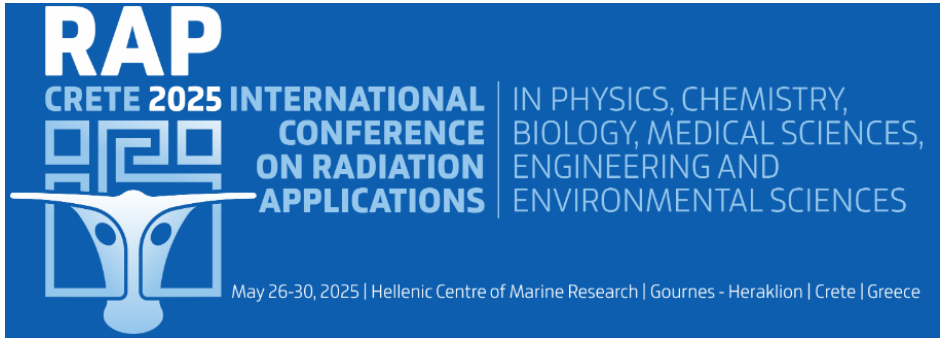


Introduction:

In space, under conditions of radiation and weightlessness, i.e. under stressful conditions, the microbiomes of space stations and humans change. Already on the eighth day of the flight, cosmonauts show a qualitative change in the composition of the endomicroflora - activation of the opportunistic pathogenic component of the microflora is observed in various biotopes, primarily in the gastrointestinal tract [*Ilyin et al. 2005*]. Changes in the quantitative and qualitative composition of the endogenous microflora can serve as a factor provoking the occurrence of chronic recurrent infections, allergic diseases, functional intestinal disorders, etc. [*Ilyin 1996*]. Such changes recorded in the composition of the endomicroflora are explained by the influence of space flight factors on the cosmonaut's body, primarily the increased radiation background on board the ship.

The intestinal microbiota is an active participant in the interaction between the gastrointestinal tract and the central nervous system. The main transmission channel is the vagus nerve. Intestinal microbiota: 1. Participates in the synthesis of neurotransmitters, actively participates in the production of serotonin, GABA and dopamine. Therefore, an imbalance of microbiota can affect mood, brain activity and even anxiety levels. 2. Maintains communication with the brain. Microbiota encourages intestinal cells to secrete serotonin, which, through nerve signals, gives the brain "feedback" about what is happening inside. Thus, healthy intestinal microbiota helps maintain a stable mood, reduce stress levels and affects cognitive functions: memory, attention and concentration.

When the balance is disturbed decreased immunity, emotional instability, lack of serotonin directly affecting mood, anxiety becoming a constant background, problems with concentration and memory, and an increase in chronic inflammation can occur. This can lead to the development of more serious diseases, from diabetes to cardiovascular disorders.

Probiotics are commonly used to prevent and treat such diseases. It has been shown that microbial cells in a metabolically active state (in the form of a fermented milk product) have a more pronounced probiotic potential than those in an anabiotic (sublimated) form. Probiotics can be used not only as a special drug, but also introduced into food products. The use of yeast probiotics for fermented products has been most well studied. Technologies for the production of fermented milk products and baking bread in aircraft or stationary modules are already being developed.

Experiments were conducted on the outside of the ISS to assess the effect of space conditions on spore forms of bacterial and fungal microorganisms. It was found that under these extreme conditions, microorganisms can not only survive, but also retain the ability to reproduce. At the same time, most microorganisms showed increased biochemical activity, and resistance to antimicrobial drugs, in particular antibiotics, increased [*Sychev et al. 2020*]. Numerous studies of microorganisms outside and inside orbital stations have shown that their aggressiveness and resistance to antibiotics increases, and microorganism spores remain viable after months in outer space. Space samples turned out to be less sensitive to the antibiotics in question (gentamicin). On the other side, it has been found that the first generations of the fungal cultures exposed outside the ISS showed increased sensitivity to clotrimazole and ketoconazole [*Deshevaya et al., 2022*].

Research objective:
evaluate the effect of radiation on the sensitivity to drugs and the induction of antibiotic resistance mutations at probiotic and backer’s yeast cells.

Strains and materials:

Laboratory strains:

Saccharomyces cerevisiae **711a** (711a)
Saccharomyces cerevisiae **9/+ 24/+** (9.24)

Commercial strains:

Saccharomyces boulardii **CNCM-I-745** (*Sb-B*), isolated from “Enterol”, *Biocodex*, France (sertified probiotic yeast strain)

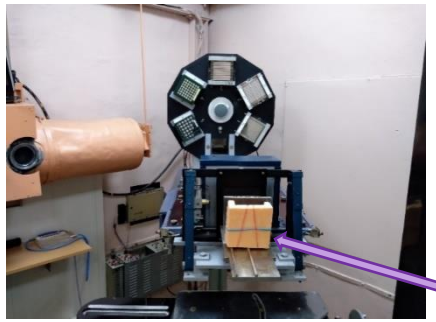
Saccharomyces cerevisiae **Puratos 1** (*Sc-P1*), one of two strains isolated from “Cosm-O-tentic”, *Puratos*, Belgium (backer’s yeast)
O-tentic® (Puratos) is a sourdough for bread. Cosm-O-tentic® was developed as part of the Puratos SpaceBakery project which focused on growing wheat in closed containers under specific conditions and circumstances, with limited resources; as dietary and food supplements; nutritional and probiotic supplements.

Genotypes:

haploid	*711a*	*MATa SUC2 mal11 mal33Δ mel gal2 CUP1 flo1 flo8-1 ade2-101 [ery221^R chl321^R oli7^R]*
diploid	*9.24*	*MATa/MATα RAD9/rad9::LEU2 RAD24/rad24::URA3*
diploid	*Sb-B*	*MATa/MATα SUC2 hxt2 hxt1 mal11Δ mal13Δ MAL33 pgm2-G1278A asp3Δ ASP1-C1445G, A1600G can1-G1445C, A1600G* [ery^R]
tetraploid	*Sc-P1*	*SUC2 hxt2 hxt1 MAL11 MAL13 MAL33 pgm2-G1278A can1-T526G, A1600G*

Irradiation of cell cultures in Eppendorf tubes with ionizing radiation was carried out at tree installations:

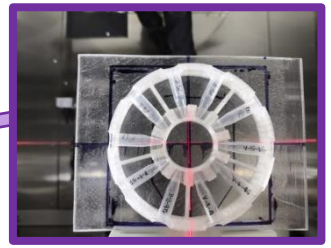
Unmodified clinical proton beams: Medico-Technical Complex (LNP JINR, Dubna) - **170 MeV proton beams, LET 0.54 keV/μm, dose rate 0.6 Gy/min**. A 52-mm Plexiglas absorber was used. Calibration was performed by ion chamber TM30013 (PTM UNIDOS-E).



Secondary beam of the phasotron

Irradiated samples Eppendorf test tube stand

X-rays: SARRP, Replicates Modern Radiation Therapy Systems by Xstrahl (LRB JINR, Dubna) - **Al-filter 1 mm, 130 kV, 13 mA, dose rate ~3 Gy/min**

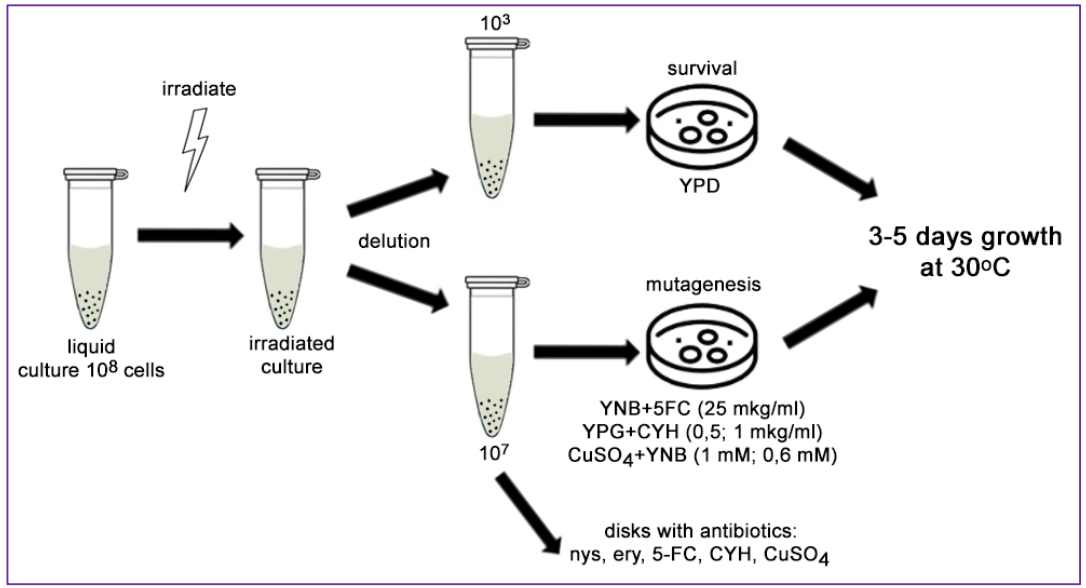


Irradiate samples Eppendorf test tube stand

X-rays: CellRad+ (LRB JINR, Dubna) - **Al-filter 0.5 mm**, **130 kV, 5 mA, dose rate ~5 Gy/min**

Irradiate samples Eppendorf test tube stand

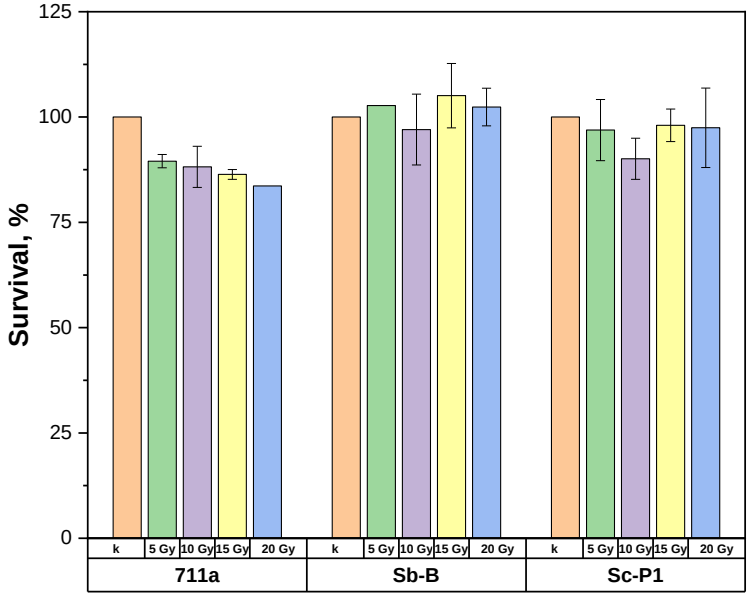
Design of experiments :



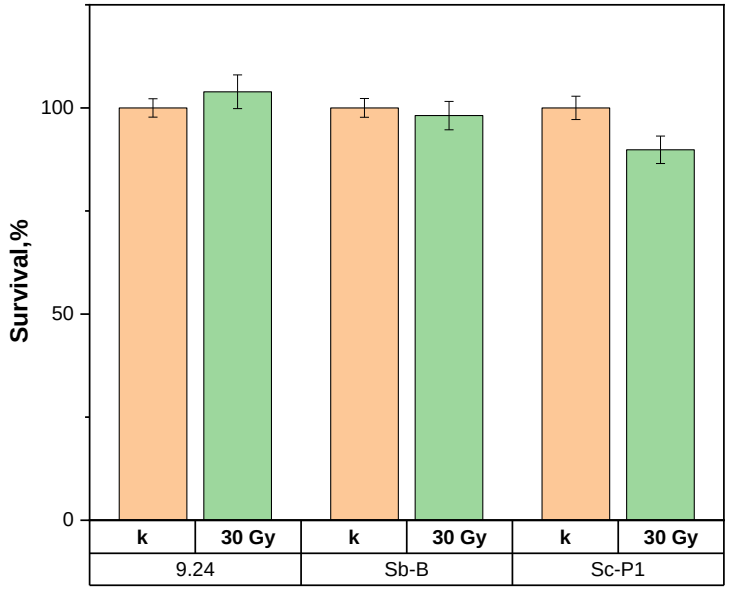
Results:

A - Survival

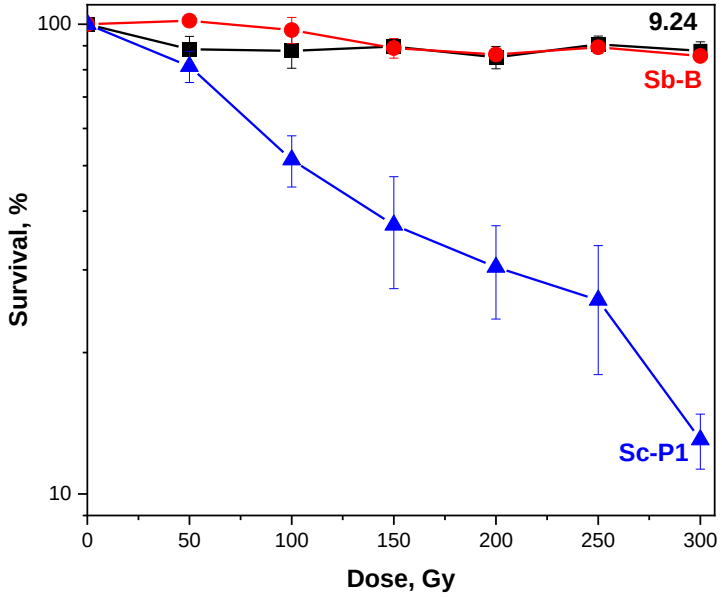
Proton irradiation (150 MeV, 0.54 keV/mkm)



X-rays irradiation (130 kV, 13 mA)



X-rays irradiation (130 kV, 5 mA)



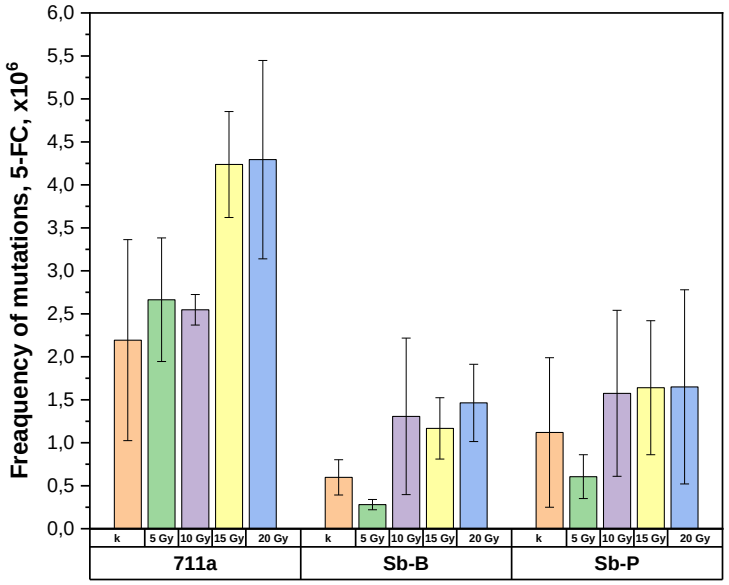
In the range of used doses, radioresistance of cells for all strains is high and the difference is observed only with irradiation at higher doses.

When irradiation was up to 300 Gy, radioresistance of yeast strains were different and corresponded by their ploidy – 9.24 and *Biocodex* were diploids and *Puratos* was tetraploid. Tetraploid was more sensitive.

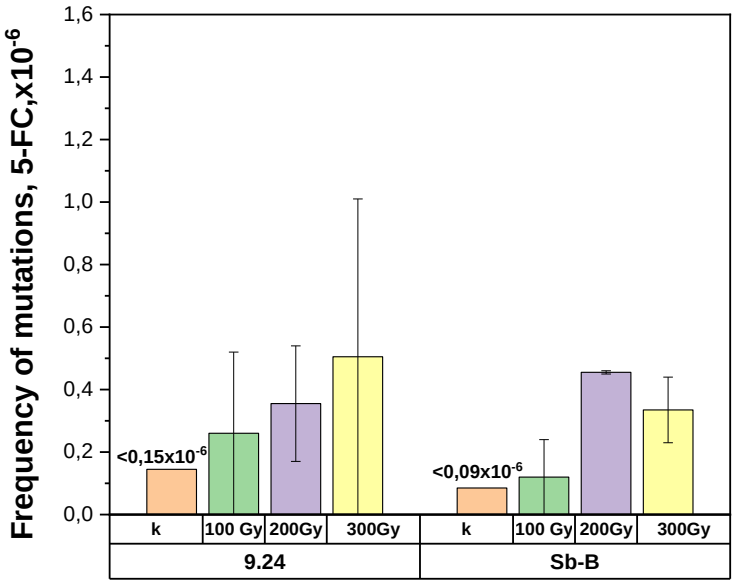
B - Mutagenesis

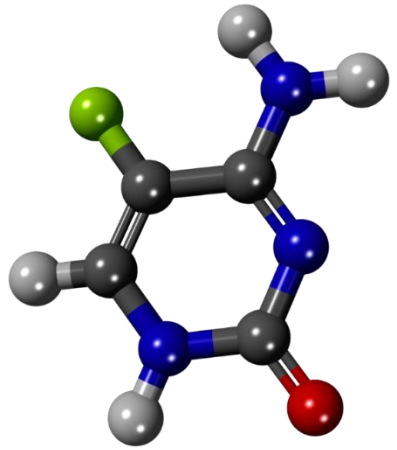
FLUCYTOSINE is a pyrimidine compound and a fluorinated cytosine (5-FC) analog exhibiting antifungal activity. After penetration into the fungal cells, flucytosine is deaminated to its active metabolite 5-fluorouracil. 5-fluorouracil replaces uracil during fungal RNA synthesis, thereby inhibiting fungal protein synthesis. Resistance to 5-FC may be acquired through point mutations in *FCY1*, a cytosine deaminase or *FCY2*, a purine-cytosine permease [*Jund, Lacroute, 1970*].

Proton irradiation (150 MeV, 0.54 keV/mkm)

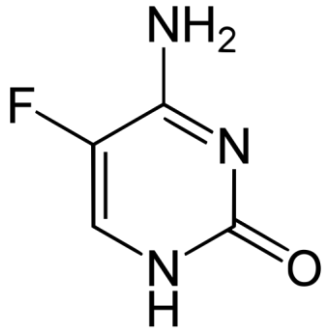


X-rays irradiation (130 kV, 5 mA)





flucytosine

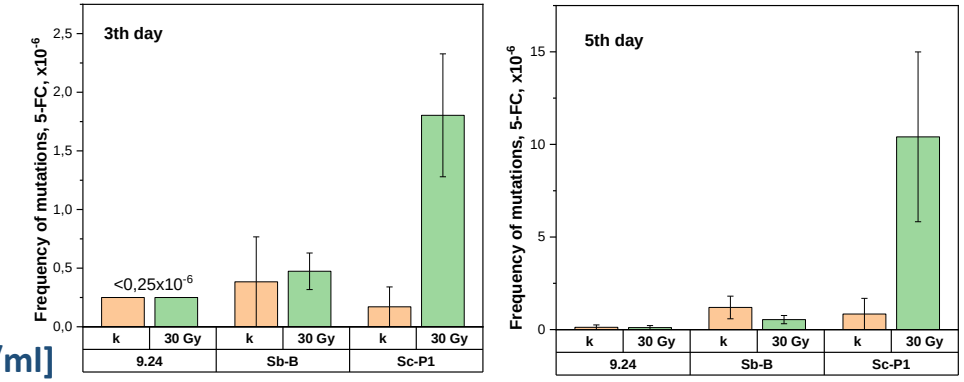


In the haploid strain 711a, 5-FC-mutation induction was observed at YNB+25 µg/ml 5-FC. In polyploid strains the mutation frequency is extremely low and is induced by protons and X-rays at low frequency, since their manifestation requires a process of homogenization, such as recombination.

X-rays irradiation (130 kV, 13 mA)

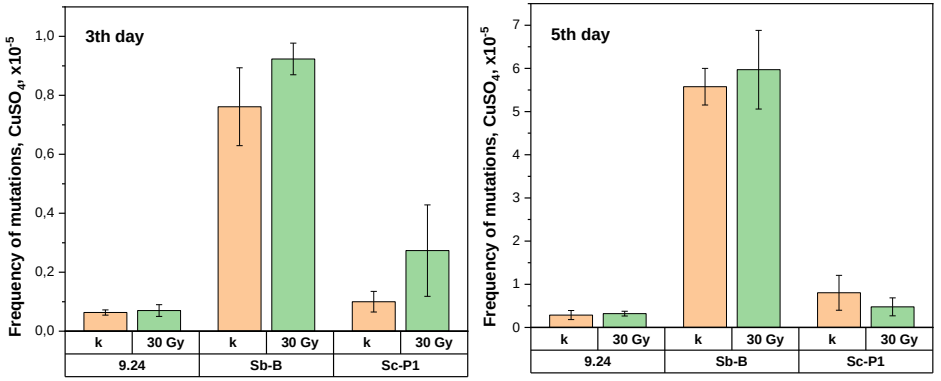
Antibiotic sensitivity can be determined by several genes that differ in their sensitivity. So we calculated the number of mutants at different times: on the third and fifth days after irradiation. Colonies were selected and minimal inhibition concentration (MIC) was determined. The more resistant mutants appear first, the more sensitive ones grow more slowly.

5-FC [25 µg/ml]



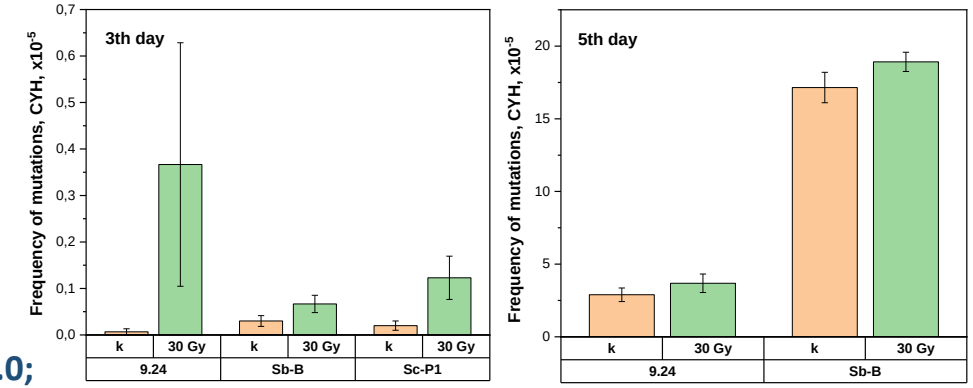
Sensitivity to **5-FC** is determined by two genes *FCY1* and *FCY2,* which mutate mainly due to point mutations, base pair substitutions.
$MIC_{5\text{-}FC}$ was ~20 µg/ml for Sb-B and SC-P1.

$CuSO_4$ [2.4; 1.0; 0.6 mM]



Resistance to **copper** is determined by the number of *CUP1* gene copies as a result of recombination events, and depends on the original copy (Sb-B – 5 copies, Sc-P1 – 2 copies).
MIC_{cyh} was 0.6 mM for Sb-B and ~0.15 mM for Sc-P1.
We select mutants at different concentration of Cu^{2+} for different strains (9.24 – 2.4 mM, Sb-B – 0.6 mM, Sc-P1 – 1.0 mM).

CYH [1.0; 1.0; 0.5 µg/ml]



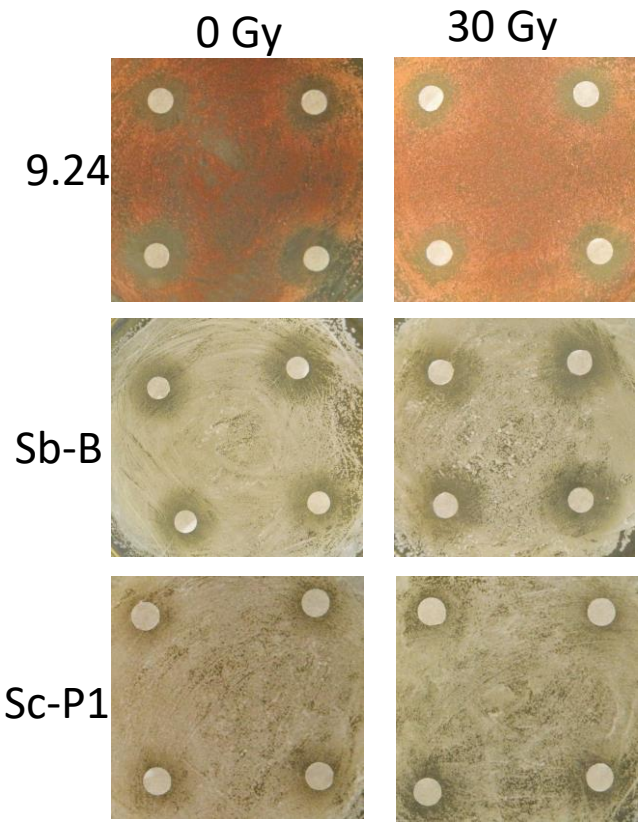
CYCLOHEXIMIDE is a potent inhibitor of eukaryotic protein synthesis. Resistance to cycloheximide was located at different loci. Region of resistance for *crl* mutants – 1-2.5 and 1-5 µg/ml. Mutations in *cyh3* and *cyh10* were isolated as being able to grow on 1,0 µg/ml. Mutations in *CYH2* gene coding ribosomal 60S subunit protein L28 were isolated as being able to grow on 10 µg/ml cycloheximide [*McCusker, Haber, 1988*].
MIC_{cyh} was 0,5 µg/ml for Sb-B and Sc-P1.
MIC_{cyh} was 2 µg/ml and 5 µg/ml for small and large mutant colonies correspondently.

Antibiotic sensitivity (disk array)

Ionizing radiation: X-rays (130 kV, 13 mA)
Agent: cycloheximide, erythromycin, nystatin, flucytosine, chloramphenycol and also $CuSO_4$

Drug	Strain	Concentr.	Death zone, mm	
			0 Gy	30 Gy
CYH	9.24	15 mkg/ml	11,25±0,19	11,21±0,18
	Sb-B		13,46±0,46	12,46±0,31
	Sc-P1		10,46±0,23	10,5±0,19
5-FC	9.24	400 mkg/ml	17,93±0,4	18,89±0,35
	Sb-B		18,27±0,77	18±0,72
	Sc-P1		14,79±0,3	15,57±0,5
$CuSO_4$	9.24	70 mM	7,59±0,14	8,36±0,19
	Sb-B	38 mM	7,34±0,14	8,0±0,3
Nys	9.24	2x100 units	10,83±0,6	12,86±0,81
	Sb-B		18,84±0,5	17,92±0,71
	Sc-P1		13,71±0,52	13,63±0,45
Ery	Sb-B	100 mg/ml	18,71±0,51	18,63±0,27
	Sc-P1		25,09±0,31	25,42±0,3
Chl	Sc-P1	40 mg/ml	7,17±0,12	7,13±0,09

CYH



no changes in susceptibility to the antibiotics used were observed in the indicated dose range

Conclusions:

- ✓ Radioresistance and mutability of the yeast strains were different and corresponded by their ploidy – laboratory and *Biocodex* strains were diploids and *Puratos1* was tetraploid. The last of them was more sensitive, at a dose of 300 Gy, survival rate decreases almost 10 times.
- ✓ Test of 5-FC-mutations has shown that generating drug-resistance through point mutagenesis is not very effective.
- ✓ The increased frequency of *CUP1* resistance in Sb-B reflects the multicopy nature of the gene in this strain.
- ✓ The large number of genes determining sensitivity to cycloheximide increases the frequency of mutations, but protons and X-rays do not induce effectively mutations within doses up to 30 Gy.
- ✓ Protons and X-rays did not change susceptibility to the antibiotics used (cycloheximide, erythromycin, nystatin, flucytosine, chloramphenycol and also $CuSO_4$) in the indicated dose range.