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Survival and changes in morphology, mitotic and metabolic activity of yeast *Candida guilliermondii* exposed to X-irradiation

L Navasardyan[1], S Marutyan[1], K Hovnanyan [2] & A Trchounian[1]*

[1]Department of Biochemistry, Microbiology, and Biotechnology, Faculty of Biology, Yerevan State University, 1 Alex Manoogian, Yerevan 0025, Armenia

[2]Institute of Molecular Biology, National Academy of Sciences of Armenia, 7 Hasratyan Str., Yerevan 0014, Armenia

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The investigation of survival and vital activity of lower eukaryotes – yeasts has a great interest under the influence of X-radiation because yeasts are widely used in radiation biology; they are convenient model for investigating of radiation-induced inactivation and further repair mechanisms of higher eukaryotes, and the mechanisms of X-rays influence on them are still unclear. The investigation of survival and vital activity of yeasts *Candida guilliermondii* exposed to X-irradiation has shown that the yeasts were characterized by the sigmoid curve of survival, and the LD_{50} was 720 Gy. After X-irradiation their growth kinetics was delayed on 2 h in comparison with non-irradiated yeasts, the ability of colony forming units in stationary growth phase was decreased approx. 3 fold. In the population of X-irradiated yeasts fibered and giant cells appeared, as revealed by electron microscopy. The vitality of non-irradiated yeasts was 56%, X-irradiated yeasts did not have the ability for fermentation, and after post-radiation incubation, and the vitality of yeasts was partially recovered; although it was significantly lower than in non-irradiated yeasts (9.7%). Thus, X-irradiation resulted the fall of mitotic and metabolic activity of yeasts. The obtained data can be used in radiation biology for developing methods of anti-radiation protection of living organisms.

Keywords: Fluorescence, NADP(H), Survival, X-irradiation, Yeast *Candida*.

Nowadays, connected with human activities, scientific and technological progress, with the increase of natural disasters and technogenic environmental pollution, the living organisms are influenced by ionizing radiation; the latter is a powerful mutagenic and cancerogenic factor[1]. So an actual problem is the identification of molecular-cellular mechanisms of influence of ionizing radiation. The investigation of survival, mitotic and metabolic activity of lower eukaryotes – yeast cells, under the influence of X-radiation, is of interest because yeasts are widely used in radiation biology, biochemistry, and biotechnology. Yeasts are considered as a convenient model for radiobiological investigations and have been used for this purpose for a long time[2-4]. This contributes to the fact that yeasts are single celled eukaryotic organisms; they can quickly multiply under the controlled conditions. The concept of a free living unicellular organism is very valuable because it should be a good model for the study of intracellular processes. This is because a true free living organism will lack cell to cell signaling and other communication phenomena that would be expected for cells from tissues of multicellular organisms[5]. From this point of view, of course, the biochemical investigations of radiation effects on yeasts are not novel. But in radiobiology, the species of yeasts *Saccharomyces* are widely used, and in the available literature, there are only a few data about the yeasts *Candida*. Moreover, there are no data with *Candida guilliermondii* irradiated by X-rays. These yeasts were of great interest due to their unprecedented radio-stability. Thus, for different yeasts the average LD_{50}= 300 Gy)[6] while according to the received datas by us Candida guilliermondii yeast LD_{50} = 720Gy, i.e. these yeast are 2.5 times radiostable in comparison with yeasts. Actually, such a great radio-stability of low eukaryotes makes them of great interest for radiobiology, biochemistry, and biotechnology. On the other hands, yeasts are similar to higher eukaryotes by their division features: yeasts are divided by mitosis[7], such as growing and quickly regenerating tissues of higher eukaryotes. So, the

*Correspondence:
E-mail-Trchounian@ysu.am

knowledge of molecular mechanisms of such high radio-stability of yeasts cells should allow developing the appropriate approaches for radio-protective events for irradiated with ionizing radiation cells of higher animals and humans.

Then, the yeasts *C. guilliermondii*, pathogenicity, virulence, and taxonomy of which are being investigated in recent years for details, are considered uncommon yeasts, the incidence of which appears as low even among compromised hosts. Although these yeasts show a reduced innate virulence compared with *Candida albicans*, its role as an agent of serious pathologies (mostly fungaemia and deep-seated infections in cancer patients) has been emphasized throughout the literature[8]. Furthermore, these species have appeared especially notable for its greater propensity to express multidrug resistance than other organisms of the genus *Candida*. Therefore, the investigations of yeasts *C. guilliermondii* are very important for suppressing of their survival and preventing their development during the combined and complex pathologies.

The mechanisms of influence of X-radiation on yeasts *C. guilliermondii* and further repair processes are still not clear. The investigation of radio-resistant strains of yeasts is not novel; they can be very significant for understanding the abilities to live organisms to resist in unfavorable conditions of environment, such as X-radiation.

The most characteristic manifestations of the general reaction of yeast cells to ionizing irradiation are disorders of cell division, atypical cell shapes, and colonies. It has been shown that after irradiation of cells with sufficiently high doses they lose their ability to divide, but stay viable and have the common ability of synthesis of cellular components, which cause the formation of giant cells[9]. Ionizing radiation influences also on many processes of yeast cells vital functions, the first of all are effects on the survival, the colony formation, and vitality of yeasts[10]. (The term "vitality" for microorganisms means the ability of fermentation. This describes many characteristics and abilities of microbial cells associated with their metabolic activity and stability against stresses[11]. For studying the cells vitality a large number of quantitative methods have been developed, including measurements based on the intracellular content of different components or manifestation of metabolic activity or gravimetric analysis of the course of fermentation. In fact, none of these methods have found with widespread application for determining the vitality of yeast cells. Indeed, sensors using intracellular fluorophores, such as NAD(P)H, have been developed[12]. A novel application of NAD(P)H-dependent fluorescence monitoring for determination of yeast vitality has been suggested[13]. The method is based on the monitoring of intensity of NAD(P)H fluorescence during a forced transition from aerobic to anaerobic conditions. The relative fluorescence increase (FI_{rel}) change and its rate (dFI_{rel}/dt) during this transition were evaluated as a measure of yeast vitality. We used this method to determine the vitality of non-irradiated, X-irradiated and repaired yeasts *C. guilliermondii*.

The aim of this study was to determine the features of survival, mitotic and metabolic activity of yeasts *C. guilliermondii* irradiated with X-rays, as well as to study electron microscopy and to investigate the vitality of yeast cells after X-irradiation and post-radiation reparation.

Materials and Methods

Yeasts, their growth, and survival

The objects of investigations were the yeast cells *C. guilliermondii* NP-4 (the lab wild-type strain). Yeast biomass was obtained by their growth in liquid medium (0.2 mM NH_4HPO_4, 0.5 mM $(NH_4)_2PO_4$, 0.6 mM K_2SO_4, 0.8 mM $MgSO_4$, 100 mM glucose, pH 5.5) on a shaker (200-250 rpm), which provided necessary aeration. The yeasts were grown at 30°C, under 4000 Lux of light, during 24 h. The yeast biomass was isolated by centrifugation (3500 g, 10 min). The amount of biomass was determined by a spectrophotometric method using the spectrophotometer GENESIS 10S UV-VIS (Thermo Fisher Scientific Inc., USA) at a wavelength of 590 nm.

Yeast survival was recorded after 24 h of incubation at 30°C in the liquid cultural medium. A comparative study of growth kinetics and colony formation of yeasts, exposed to X-irradiation, was done for the first 24 h of post radiation incubation. The parameters of growth, duration of latent (lag) period and growth specific rate (μ), were determined by hourly measurements of optical density (OD) of the culture medium with non-radiated and X-irradiated yeasts. μwas determined at the interval when the logarithm of OD varied linearly during the time by equation lg2/t, where t is the doubling time of OD; duration of latent (lag) phase (t_d) was determined graphically. The number of surviving yeast cells was determined by titration on Petri plates with medium 2% agar-worth[14]. The delayed effect of colony forming was also taken into account[15].

X-irradiation of yeasts

For investigation of survival and vital activity, the yeasts were exposed to X-irradiation by X-machine Dron-3 (Russia) with X-tube BSW-29Mo ($\lambda = 0.71\times 10^{-8}$ cm) or BSW-25Cu ($\lambda = 1.54 \times 10^{-8}$ cm), at dose interval of 8.6-1000 Gy. The further investigation of kinetic parameters of growth, colony forming and survival of yeasts was realized by X-irradiation of cells with the dose of 300 Gy (U = 35kV, I = 15 mA, duration of irradiation 30 min).

Scanning electron microscopy of yeast

For electron microscopic investigations of non-irradiated, irradiated and repaired yeast suspensions were fixed in 2.5% glutaric aldehyde solution in 0.1 M cacodylate buffer[16]. Post-fixation was realized by 1% solution of OsO_2 in the same buffer at room temperature during 1 h. Scanning of preparations was realized by the electron microscope Tesla BS-301 (Tescan, Czech Republic). Analysis of images was done using software "VideoTest Structure 5. Nanotechnology"[17].

Vital activity of yeasts

The Vital activity of yeasts was measured by registration of intracellular NADP(H) fluorescence intensity as described[13] using a fluorescence spectrophotometer FluoroMax^{TM} (SPEX Industries Inc., USA). The vitality of yeasts was determined as a relative NADP(H) fluorescence intensity increase (FI_{rel}, %) in case of forced aerobic (AE)-anaerobic(AN) transition by the equation: $FI_{rel} = (FI_{AN} - FI_{AE}) \times 100$.

The aerobic-anaerobic forced transition of yeasts was provoked creating alternative aerobic and anaerobic conditions in cell suspensions. This was carried out by sparging either air or nitrogen (0.1 L/min) into the cuvette with suspended yeasts. The $FI_{340/440}$ signal was first recorded on-line every 0.5 sec during aerobic conditions until a constant output signal was reached (FI_{AE}). Then the air flow was switched to nitrogen which led to the step-wise increase of the $FI_{340/440}$ signal due to oxygen depletion in cells (FI_{AN}).

Reagents and data processing

The reagents of analytical grade were used throughout. Each experiment was repeated at least three times; errors were given in tables and error bars were presented on figures. Standard errors such as standard deviation were calculated using the appropriate function of Microsoft Excel 2013. The changes were validated by calculation of Student's validity criteria (P); the differences between experiments (irradiated and non-irradiated cells) were valid if $P < 0.05$.

Results

Influence of X-rays on yeast survival

It has been known for a long time, that the sigmoid survival curves are typical for eukaryotic organisms, and the exponential curves for prokaryotes. Yeasts are eukaryotic organisms though lower eukaryotes[5]. But from this point of view, there are some differences among different species of yeasts. Thus, the survival curve shape of yeast cells is dependent on their genome ploidy: for haploid yeast cells, the exponential survival curves are typical. For diploid yeast species the sigmoidal survival curves are typical, such as for higher eukaryotes, and by this point of view the investigated by us yeasts also may be a convenient model to investigate the survival patterns of higher eukaryotes. The data obtained have shown that for the yeasts *C. guilliermondii* decrease of viability is seen while raising the X-irradiation dose, and the survival curve has a sigmoidal shape: in case of low doses (up to 35 Gy) of X-irradiation the yeasts survival maintained within the 100%, and in case of higher dose (up to 1200 Gy) it is decreasing and causing initial platform. So, for yeasts *C. guilliermondii* the sigmoidal survival curve is typical (Fig. 1). Such dependence of survival was observed in cases, when the cells have many (more than one) sensitive targets, such as DNA or chromosomes, or there is functioning a mechanism of radiation-induced damages repair. This mechanism of cell survival is called "The repairable-conditionally repairable damage (RCR) model"[18] and can describe cell survival for a wide range of affected factors. It separates the probability of inducing damage and the

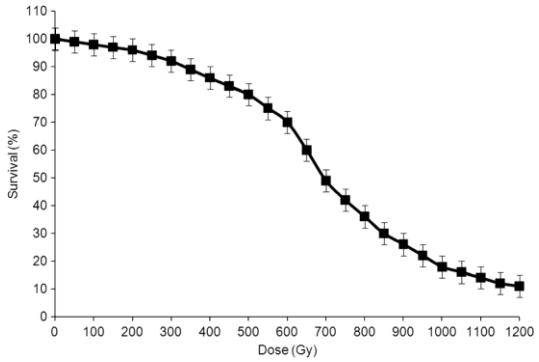

Fig. 1 — The dependency of survival of yeasts *C. guilliermondii* NP-4 on the dose of X-irradiation (n=5). For details see Materials and methods.

probability of cellular repair, enabling these effects individually. The RCR model assumes that a cell can survive either by not receiving any damage or by correctly repairing the acquired damage[19]. Sigmoid curves of survival are typical for higher eukaryotes[20]; it is also characteristic for some fungi, particularly for yeasts[21]. It can be suggested that yeasts have a certain mechanism of damages reparation so they can be a model in radiation biology for investigating the influence of radiation on the physiological functions of higher organisms.

Analysis of *C. guilliermondii* survival has shown that LD_{50} of these cells was 720 Gy. There is the report that for yeasts *Saccharomyces cerevisiae*, which is closely related to *C. guilliermondii*, LD_{50} is 300 Gy[6]. The results obtained indicated that *C. guilliermondii* cells are 2.5 fold radio-stable than *S. cerevisiae* (Table 1). Such high radio-stability can be explained by the existence of an effective mechanism of DNA reparation in cell nuclei and by the existence of quite solid cell wall, which consist of protein-carbohydrate complex, as shown by a screening of X-rays, by features of radical-quenchers, particularly histones[22].

Changes in growth and morphology of yeast cells under the influence of X-irradiation

Under the influence of X-irradiation on yeast cells the changes in growth parameters as well as in the ability of colony forming were observed. As it was seen in Fig. 2, t_d of irradiated yeasts was 7 h, which was 2 h longer than in case of non-radiated cells. At the end of lag-phase, adapting to environmental conditions, both non-irradiated and irradiated cells started dividing actively and, actually, crossed to exponential (log) phase of growth.

Such delay can be explained by genetic changes in irradiated yeasts, as a result of which the cell cycle was violated, particularly the phase of mitosis was delayed. As a result of this, the delay of mitotic activity was observed. The cause of the delay of mitosis can be the delay of transition of cells into a G_2 phase of cell cycle, or the G_2-block, as it was called[22]. This was characterized by the fact that irradiated cells passed to prophase with typical rate and accumulated in this phase: further cell division was delayed. The delay may also be characterized by the chromosomal aberrations that occur in the cells under the influence of radiation; despiralization of chromosomes and turning them into interphase chromosomes, so the cells "return" from prophase to interphase; disorders of protein synthesis involved in mitosis, especially in the synthesis of centrioles[23].

During the first two hours after irradiation, due to the operation of reparation system of cells, a part of damages were repaired, and cell population entered into the log-phase of growth (Fig. 2). The stationary growth phase of radiated yeasts set again with 2 h

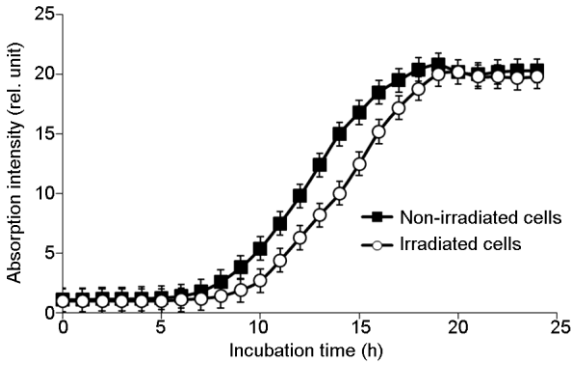

Fig. 2 — Growth kinetics of yeasts *C. guilliermondii* NP-4 before and after X-irradiation (n=3).

Table 1 — Some characteristics of growth of yeasts *C. guilliermondii* NP-4 after X-irradiation by dose 300 Gy (n=5)

Growth phases	Non-irradiated cells				Irradiated cells*			
	Colony numbers, $\times 10^9$ cell/mL	Absorption intensity, rel. unit	μ*, h^{-1}	t*, h	Colony numbers, $\times 10^9$ cell/mL	Absorption intensity, rel. unit	μ, h^{-1}	td, h^{-1}
Lag phase	0.40 ± 0.08	0.21 ± 0.01	ND**	ND	0.60 ± 0.09 *P* <0.05	0.17 ± 0.01 *P* <0.05	ND	ND
Early lag-phase (6th h of growth)	13 ± 1	0.26 ± 0.02	0.39 ± 0.04	1.78 ± 0.1	0.40 ± 0.02 *P* <0.002	0.21 ± 0.01 *P* <0.05	0.23 ± 0.02 *P* <0.01	3.00 ± 0.12 *P* <0.01
Late log-phase (16th h of growth)	27 ± 2	0.41 ± 0.03	0.55 ± 0.02	1.26 ± 0.09	10 ± 1 *P* <0.01	0.39 ± 0.04 *P* >0.05	0.37 ± 0.03 *P* <0.05	1.87 ± 0.08 *P* <0.01
Stationary phase (21th h of growth)	28 ± 3	0.42 ± 0.01	ND	ND	10 ± 1 *P* <0.01	0.40 ± 0.04 *P* >0.05	ND	ND

*For irradiation conditions see Material and Methods; μ – specific growth rate of yeast cells; t_d –doubling time of OD of the cultural medium of yeasts; *P* was calculated for the appropriate differences between irradiated and non-irradiated cells. ** Not determined

delay, although approaching the stationary growth phase the difference between the biomass amounts of the irradiated and non-irradiated cells practically disappeared (Table 1).

The data obtained in the study of the rate of colony formation in *C. guilliermondii* (Fig. 3), confirmed the results of the study of the growth kinetics of non-irradiated and radiated cells. It should be noted that in the case of irradiated cells a drop in growth rate has occurred in comparison with non-irradiated cells. Appropriate quantitative data were presented in Table 1, showing that in all phases of post-radiation incubation the differences in the rates of colony formation of non-irradiated and X-irradiated yeasts were observed. So, for non-irradiated yeast cells in early log phase of growth (the 6^{th} h of growth), an increase of colonies number of 300 fold was observed but for X-irradiated yeasts only 7 fold. In the late-log phase of growth (the 16^{th} h of growth) for non-irradiated yeasts the doubling of colonies number was observed, and in the case of X-irradiated cells, the number of colonies was increased approx. 25 fold. However, in the stationary phase of growth, there were no significant changes of numbers of colonies in both variants of yeast cells.

Thus, in comparison with the initial point of growth in stationary phase the increase of colonies number for non-irrdiatad yeasts of about 700 fold has taken place and for irradiated yeasts about 170 fold. It can be suggested that for non-irradiated yeasts the intensive growth was observed in the early-log phase of growth. For X-irradiated cells in this phase, the decrease of μ was determined, but more intensive growth was observed in late-log phase. These data confirmed the observed in irradiated yeast growth kinetics picture 2 h delay of log and stationary phases. Simultaneously, from the data of Table 2, it was evident that the rate of accumulation of biomass together with the number of colonies naturally was increased (about 3 fold). However, in the stationary phase of growth, there were no significant differences in the amount of biomasses for non-irradiated and X-irradiated yeasts. This can be explained by the presence of small amounts of rapidly growing cells or formation of giant cells in the population of irradiated yeasts. To confirm this suggestion, a comparative study of morphological changes in the X-irradiated and repaired yeast cells was realized the first time by scanning electron microscopy. In a population of intact yeasts were found rounded, oval or clavate

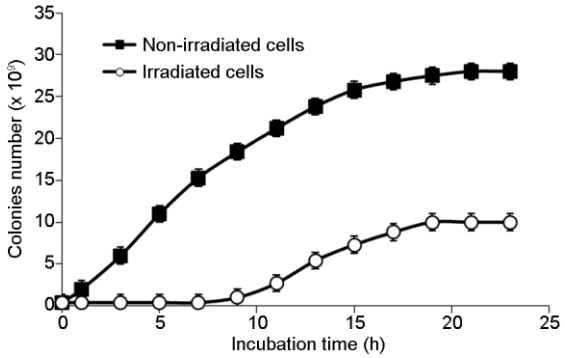

Fig. 3 — Colony forming ability of yeasts *C. guilliermondii* NP-4 before and after X-irradiation (n=5).

Table 2 — Vital ability of yeasts *C. guilliermondiii* NP-4 during forced aerobic-anaerobic transition (n=5)

Yeast cells	FI_{AE}^{*}, 10^6 cells/s	FI_{AN}^{*}, 10^6 cells/s	Fi_{rel}, %
Non-irradiated	1.1 ± 0.1	1.72 ± 0.20	56.0 ± 1.2
Irradiated (300 Gy for 30 min)	0.72 ± 0.02P <0.05	0.75 ± 0.01 *P* <0.01	0
Repaired (after 24 h of irradiation by 300 Gy for 30 min)	0.97 ± 0.03 P >0.05	1.06 ± 0.08 *P* <0.05	9.3 ± 0.8 *P* <0.001

* Forced aerobic, FI_{AE}; Forced anaerobic, FI_{AN}

shaped cells with a smooth surface (Fig. 4). After exposure to X-rays on yeast cells (Fig. 5) in the irradiated cell populations spherical, as well as fibered (A), and giant (B) cell forms were found out. Thus, the obtained results confirmed the data of growth kinetics and colony formation ability of X-irradiated *C. guilliermondii*.

The repaired yeast cells, after 24 h of post-radiation incubation in medium, contributes the reparation of cells from radiation damages, in the cell populations by scanning electron microscopy. Cells compared with control culture were found (Fig. 6). Side by side with them a number of elongated filamentous forms were stored.

Thus, the results obtained using the electron microscopy have shown that the X-irradiation led to morphological damages of *C. guilliermondii*, some of which were recovered in the post-radiation reparation process of the yeast cells.

Changes in the metabolic activity of yeast cells under the influence of X-irradiation

The viability of yeast cells before and after X-irradiation and post-radiation incubation has been determined (Fig. 7, Table 2). It was shown that the viability of the intact yeast cells was 56%.

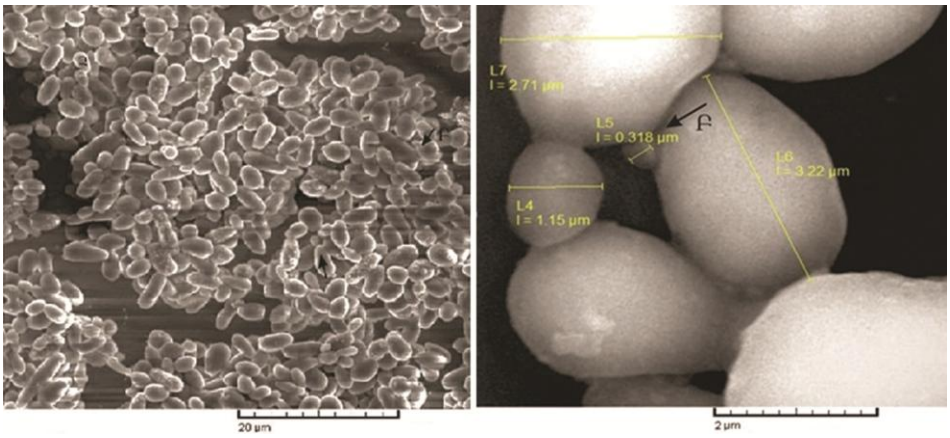

Fig. 4 — Scanning electron microscopy of non-irradiated yeasts *C. guilliermondii* NP-4(n=7). For details see Materials and methods.

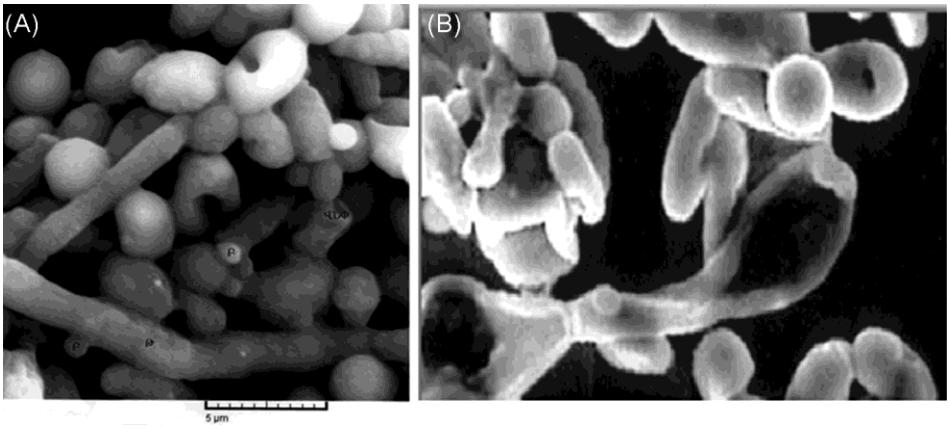

Fig. 5 — Scanning electron microscopy of yeasts *C. guilloiermondii* NP-4, exposed to X-irradiation: filamentary (A) and giant (B) forms of cells (n=7)

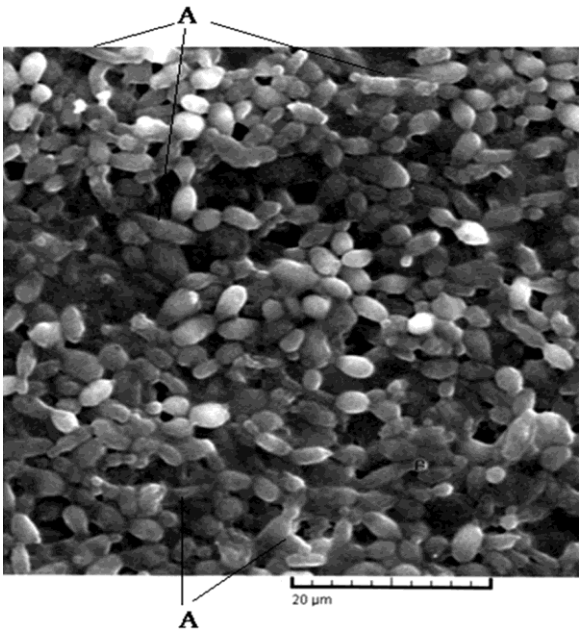

Fig. 6 — Scanning electron microscopy of yeasts *C. guilliermondii* NP-4 after post-radiation repair (n=7).

The viability of X-irradiated yeasts disappeared, *i.e.* during the forced aerobic-anaerobic transition of yeast suspension there was no any change of fluorescence of intracellular NADP(H). After post-radiation incubation yeasts viability was restored partially by 9.3%; thus it remained lower than its initial value of approx. 6 fold.

It can be concluded that under the influence of radiation the vital activity of yeast cells, including their viability, or "fermentation ability", was disrupted. Probably, under the influence of X-rays, the changes occurred in yeast metabolic process as a result of which the intensity of the fluorescence of intracellular NADP(H) was changed. During the post-radiation incubation period of yeasts the restoration, processes were taken place gradually in reparation of DNA damages, correction of structural damages of cells and recovery of different mechanisms of vital activity. As a result, in yeast cells, the ability of the fermentation was restored, although it was much lower in comparison with intact cells.

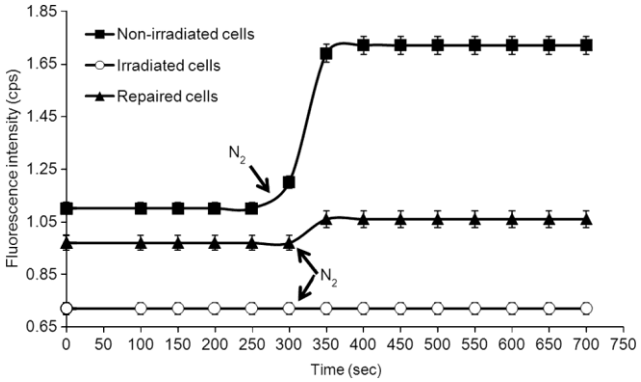

Fig. 7 — Fluorescence of intercellular NADP(H) in yeasts *C. guilliermondii* NP-4 after X-irradiation in comparison with non-irradiated yeasts (n=5). N_2 was added during incubation (arrows).

Discussion

The investigation of yeast cells *C. guilliermondii* survival exposed for X-irradiation has shown that in yeasts the decrease of survival was observed under the influence of X-rays by sigmoid curve depending on radiation dose. This was not typical for lower eukaryotes[20]. Sigmoid curves of survival were observed in the cases when a mechanism of reparation of radiation-induced damages operated in the cells. Under the influence of radiation, damages occurred, inactivating the cells. The reparation system of a cell decreased the probability of cell inactivation due to damages. Together with the radiation dose increase, the efficiency of reparation system decreased, *i.e.* under the influence of radiation the reparation system itself was damaged, or the damages were accumulating which could not be repaired. Thus, the survival curve of yeasts has taken the sigmoid form, and besides the initial flexion of curve showed the sensibility of the cells, having non-damaged, complete mechanism of reparation of damages, and the final flexion corresponded to sensitivity of the cells, which reparation system was fully inactivated[20]. Taking into account that sigmoid curves of survival are typical for higher eukaryotes[20], it can be suggested that survival of yeasts *C. guilliermondii* were similar to them and could be used as a convenient model in radiation biology for investigations of the influence of radiation on the physiological functions of higher eukaryotes.

It is of interest that LD_{50} for *C. guilliermondii* is 720 Gy, which is about 2.5 fold higher than for yeasts *Saccharomyces*[6]. Such a high radio-stability can be connected with specific molecular processes in cells, and the study of such possibilities could be perspective for developing of possible methods and ways of protection of higher eukaryotes.

It is well known that under the influence of X-irradiation in DNA different structural damages take place[12,23]. These damages include the violations of nitrogen basis and breaks of DNA strands. It is possible also the appearance of crosslinks between DNA molecules, as well as between DNA and proteins[6]. The single-stranded breaks of DNA in the process of post-radiation incubation can turn into double-stranded breaks as a result of so-called "disrepair"[12]. The decrease in survival curves (see Fig. 1) corresponds, apparently, to the period of maximal appearance of breaks, when the cell survival should be minimal. This relates to elongated lag-phase of growth of X-irradiated yeasts. The further incubation of damaged cells flows with DNA reparation, as a result of which the rate of biomass accumulation gradually increases and about to 20^{th} h of growth the irradiated yeasts reach to non-irradiated ones according to the amount of accumulated biomass.

At the same time, the colony forming ability was gradually recovered (see Table 1). But in this case, even after 20 h incubation, the irradiated cells conceded non-irradiated cells about 3 fold. It is possible that after radiation, as a result of DNA reparation, a small amount of colony forming cells was increased that was characterized by a rapid growth. It is also possible that the high value of biomass amount for X-irradiated yeasts at the end of incubation is not the result of the intensive growth of cells, but the result of the formation of giant cells.

Conclusion

Based on the results obtained it can be concluded that survival of yeasts *C. guilliermondii* exposed for X-irradiation has a sigmoid form depending on the X-rays dose; LD_{50} of the yeasts exposed to X-irradiation is 720 Gy, and under the influence of X-irradiation a decrease of mitotic and metabolic activity of the yeasts was occurred that partially was recovered after post-radiation reparation. The obtained data can be used in radiation biology and biochemistry for developing methods of anti-radiation protection of living organisms, such as for developing the vaccine.

We hypothesized that in yeast cells *C. guilliermondii*a stable mechanism of repair of X-radiation induced damages is working. It can be connected to the newly synthesized protector proteins. It is known that under any extreme conditions the

special proteins are synthesized in cells, which are known as thermo-shock proteins[24,25]. It is possible that the synthesis of proteins of this family occurs as a result of radiation stress. This fact is recorded in our investigations as a result of which it has been shown that new sub-fractions are seen in the electrophoregram of water-soluble proteins of yeasts *C. guilliermondii* after X-irradiation and post-radiation repair. Our further investigations would be continued towards the study of water-soluble proteins fractional composition and its changes under influence of X-irradiation and post-radiation repair period.

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