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The study of the influence of exogenous factors on Saccharomyces cerevisiae DNA spheroplast

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Abstract. The possibility of fixing genotoxic changes in Saccharomyces cerevisiae yeast under the influence of physicochemical factors was evaluated using the DNA-comet assay. Peripheral blood leukocytes of mice subjected to similar effects were used as an object of comparison. The data obtained showed that under the action of both the alkylating agent and X-ray irradiation, the changes were more pronounced in experiments with leukocytes of the blood of mice. At a methyl-methane sulphonate concentration of 10 mM, the comet tail length in mouse peripheral blood leukocytes was about 40 μm, while the yeast spheroplasts tail length of a comet was 0.16 μm. The DNA content in the tail of the comet did not exceed 5 % for yeast after treatment with an alkylating agent at a concentration of 40 mM, which is 10 times less than in peripheral blood leukocytes of mice under similar conditions. Under the action of X-ray radiation, the length of the comet's tail and the DNA content in it for spheroplasts of yeast also differed significantly from the leukocytes of the peripheral blood of the mouse. Thus, the comet test allows to register a significant increase in the level of DNA damage in yeast spheroplasts under the action of physicochemical factors in relatively high doses.

The DNA-comet assay is one of the most effective biomonitoring methods is the direct assessment of DNA damage in cells. This method makes it possible to identify genotoxic interplay under the conditions of cytotoxic factors action, allowing to detect single nicks, alkaline-sensitive sites, DNA-DNA, and DNA-protein crossings, as well as incomplete repair synthesis sites. Currently, the method is widely used in studies of the genotoxicity of ionizing radiation, pharmaceuticals, industrial chemicals, and clinical studies [1-3]. The main objects of these studies are people or animals. At the same time, there is practically no mention of its applicability for lower eukaryotic organisms, including yeast. In this regard, the aim of the work was to study the applicability of the DNA-comet assay for analyzing the detection of DNA damage in *Saccharomyces cerevisiae* yeast cells.

It is widely thought that the length of the comet's tail is directly related to the size of the fragments and is proportional to the number of single-strand breaks and alkali-sensitive sites that occur in DNA as a result of the action of various damaging agents. The most obvious parameter characterizing the degree of DNA damage is the percentage of DNA content in the tail of the comet, or the ratio of the amount of DNA in the tail of the comet to its total intensity expressed as a percentage. Taking this into account, two parameters to assess the genotoxic effects of various physicochemical factors on the DNA of spheroplasts of yeast: the length of the comet's tail and the percentage of DNA in the comet's tail were used in current experimental work.

The alkaline version of the DNA-comet assay proposed by Singh et al. in 1988 was taken as the basis [4]. The main difficulty with the use of *Saccharomyces cerevisiae* in the comet test was the need to destroy the cell wall in order to obtain spheroplasts. The cell wall of the yeast is difficult to lyse, since it is represented by a rigid structure and makes up about 25% of the dry matter of the cell. In the literature, cell wall lysis is proposed to be carried out by means of enzymes. To obtain spheroplasts of the yeast *Saccharomyces cerevisiae*, the snail enzyme Helix Pomatia was used in current experiments [5].

A number of publications using the DNA-comet assay on yeast cells have discussed that the last-mentioned have a very low DNA content. The haploid yeast nucleotides contain only about 13 million base pairs in contrast to the mouse leukocyte nucleotides that contain about 3,300 million [6]. Miloshev, comparing comets of the *Saccharomyces cerevisiae* yeast with comets of the peripheral blood of the mouse, asserted that the yeast core has more compacted structure than the leukocyte nucleus of the mouse because the yeast cell's chromatin is much more compact and the comet's tail has a fragmented structure [7].

The DNA-comet assay was used to evaluate the sensitivity of yeast spheroplasts to the action of various physicochemical factors (alkylating agent and ionizing radiation) compared with the sensitivity of peripheral blood leukocytes of mice under similar experimental conditions.

From the data presented in figures 1 and 2, it is shown that the cometary tail length is reliably recorded at a methyl-methane sulphonate (MMS) concentration of 10 mM for yeast spheroplasts ($p < 0.05$) and at MMS concentration of 0.2 mM for mouse peripheral leukocytes ($p < 0.01$).

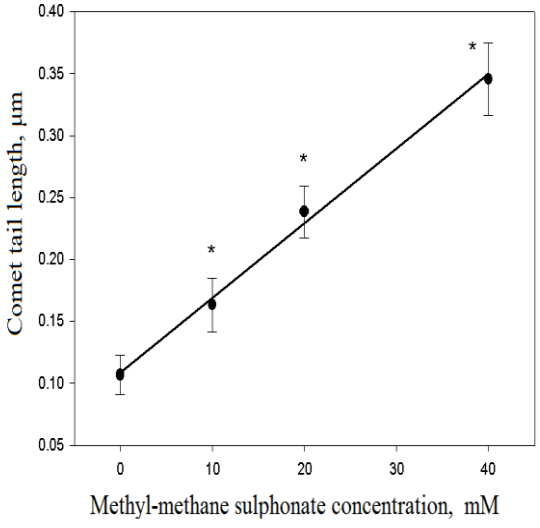

Figure 1. Comet tail length in yeast spheroplasts after MMS treatment at various concentrations.
* $p < 0.05$ - significant differences from the control by Student t-test.

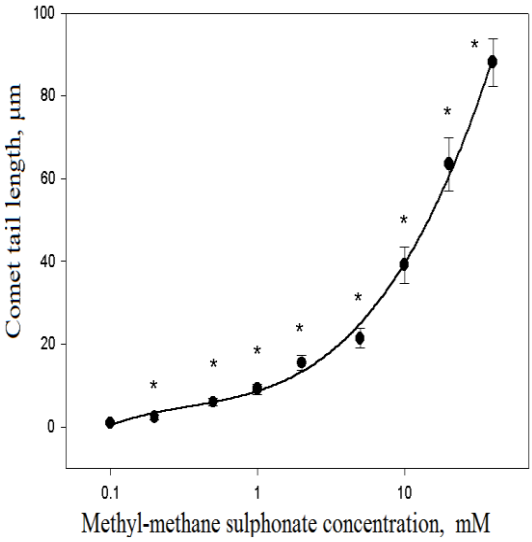

Figure 2. Comet tail length in mouse peripheral blood leukocytes after MMS treatment in various concentrations.
* $p < 0.01$ - significant differences from the control by Student t-test.

It should be noted that with an MMS concentration of 10 mM, the cometary tail length in mouse peripheral blood leukocytes is about 40 µm, unlike yeast spheroplasts, the comet tail length of which is significantly less - 0.16 µm.

The percentage of DNA in the tail of a comet under the action of different concentrations of the alkylating agent on yeast spheroplasts and mouse peripheral blood leukocytes is presented in figures 3 and 4.

It was found that after treatment of yeast spheroplasts with MMS at a concentration of 20 mM, the level of DNA damage increases significantly compared with the level of DNA damage in the corresponding control ($p < 0.02$) and amounts to about 2 %.

Compared with yeast spheroplasts, DNA lesions in peripheral blood leukocytes of mice are reliably recorded at MMS concentrations of 0.2 mM ($p < 0.01$), and at MMS concentrations of 10 mM, the percentage of DNA in the comet's tail is about 15 %.

It should be noted that the results obtained by the DNA-comet assay may depend on a number of conditions, the most critical of which are the modification of the method used, the conditions of cells treating with damaging agents and the organization of the chromatin of the studied cells.

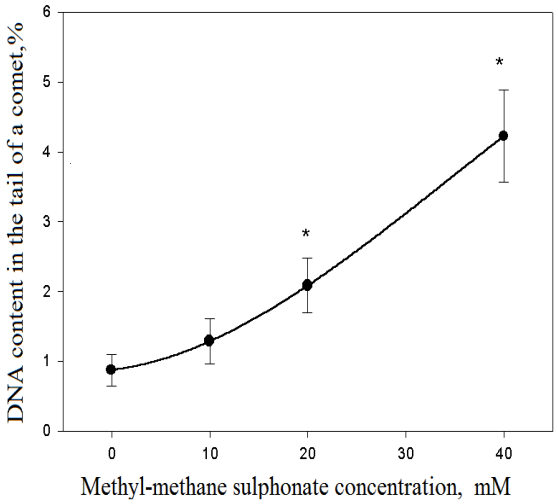

Figure 3. The percentage of DNA in the tail of a comet in spheroplasts of yeast after MMS treatment in various concentrations.

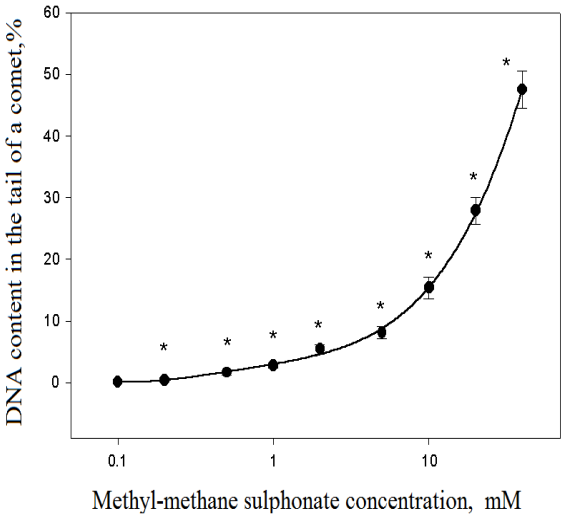

Figure 4. The percentage of DNA in the tail of the comet in the peripheral blood leukocytes of the mouse after treatment MMS in various concentrations.

In this regard, it is important for comparative analysis in addition to evaluating the sensitivity of the method (the level of the minimum reliably detectable concentration of a chemical or radiation dose) to bring the concentrations and doses corresponding to significant levels of DNA damage in the cells.

From tables 1 and 2 it follows that the DNA content in the cometary tail is 2 % after treatment of yeast spheroplasts with MMS at a concentration of 20 mM, whereas in the peripheral blood leukocytes of mice, the DNA content in the cometary tail reaches 2 % after treatment with MMS at 1 mM (20 times lower in comparison with yeast spheroplasts).

Table 1. Standard parameters of comets obtained by analyzing images of yeast cells after the action of an alkylating agent (MMS) in various concentrations.

Concentration MMS, mM	Comet tail length, μm	DNA content in the tail of a comet, %
Control	0.107 ± 0.016	0.87 ± 0.22
10	0.163 ± 0.022*	1.28 ± 0.32
20	0.239 ± 0.021*	2.08 ± 0.39*
40	0.346 ± 0.029*	4.22 ± 0.66*

* $p < 0,05$ – significant differences from the control by Student t-test.

In the next series of experiments, the genotoxic effects of X-rays were investigated. According to the literature, sufficiently high radiation doses (20 to 160 Gy) were used to study the damaging effects

of X-rays on yeast cells using DNA-comet assay [8]. Taking into account these data, the sensitivity of yeast cells spheroplasts to the effect of various X-ray radiation doses was evaluated using the DNA-comet assay.

The data presented in figure 5 demonstrate that after irradiation of yeast spheroplasts at a dose of 10 Gy, the level of DNA damage is reliably recorded ($p < 0.05$). Increasing the dose by 2, 4 and 8 times causes an even greater level of DNA damage in the cells, which increases linearly with increasing dose.

Table 2. The level of DNA damage in peripheral blood leukocytes of mice under the action of an alkylating agent (MMS) in various concentrations.

Concentration MMS, mM	Comet tail length, μm	DNA content in the tail of a comet, %
Control	0.300 ±0.11	0.05 ±0.02
	0.81 ±0.31	0.16 ±0.08
0.2	2.24 ±0.55*	0.41 ±0.11*
0.5	6.0 ±0.9*	1.63 ±0.33*
1.0	9.1 ±1.2*	2.74 ±0.39*
2.0	15.4 ±1.8*	5.40 ±0.71*
5.0	21.5 ±2.5*	8.1 ±1.0*
10.0	39.1 ±4.3*	15.4 ±1.8*
20.0	63.4 ±6.4*	27.9 ±2.1*
40.0	88.0 ±5.7*	47.5 ±3.0*

* $p < 0,05$ – significant differences from the control by Student t-test.

But the length of a comet's tail in yeast cells is increased by an insignificant amount, unlike the peripheral blood leukocytes of a mouse, the level of DNA damage in which is reliably recorded after irradiation at a dose of 20 cGy ($p < 0.01$) and at a dose of 10 Gy the tail length was about μm (figure 6).

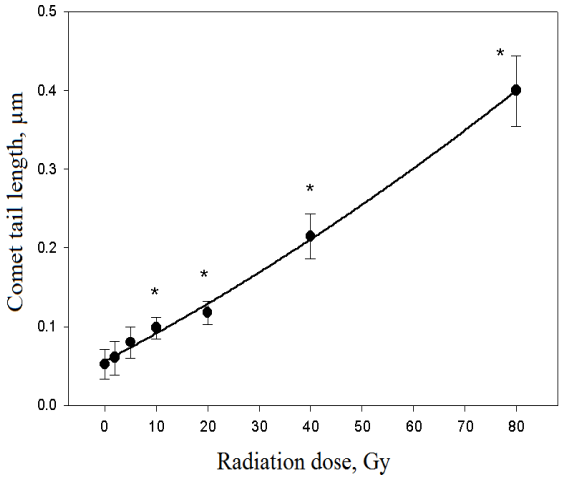

Figure 5. Comet tail length in yeast spheroplasts after the action of various doses of x-ray radiation.

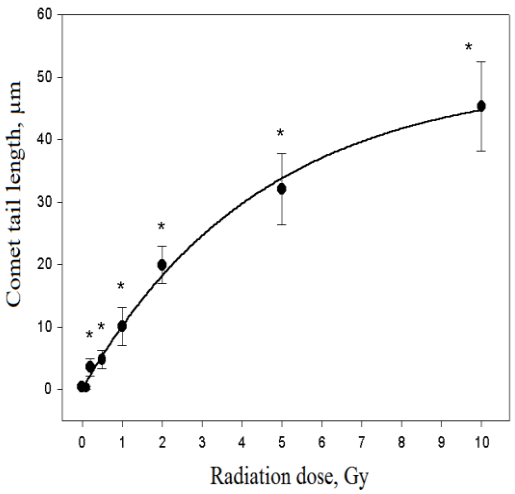

Figure 6. Comet tail length in peripheral blood leukocytes mice after the action of various doses of X-ray radiation.

Analysis of comets by the percentage of DNA in the comet tail in yeast spheroplasts and in mouse peripheral blood leukocytes (figures 7 and 8) showed that the percentage of DNA in the comet tail is reliably recorded when irradiated at a dose of 10 Gy for yeast spheroplasts ($p < 0.02$) and when irradiated at a dose of 20 Gy for mouse peripheral blood leukocytes ($p < 0.01$).

In mouse blood leukocytes the level of DNA damage increases linearly with increasing radiation dose and reaches 20 % at a dose of 10 Gy (figure 8), while the percentage of DNA in the comet tail in yeast spheroplasts is about 1 % at an X-ray radiation dose of 10 Gy (figure 7).

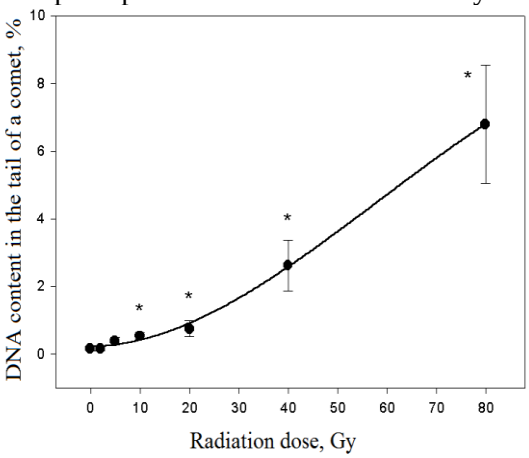

Figure 7. The percentage of DNA in the tail of a comet in spheroplasts yeast after the action of various doses of X-ray radiation.

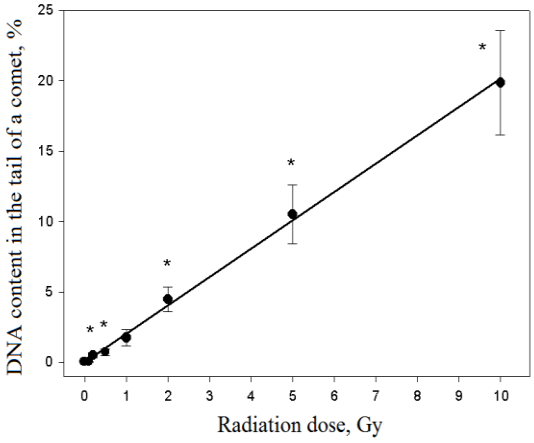

Figure 8. The percentage of DNA in the tail of a comet in mouse peripheral blood leukocytes after various doses of X-ray radiation.

Tables 3 and 4 present the standard parameters of comets, obtained by analyzing images of yeast cells and mouse peripheral blood leukocytes after irradiation with ionizing radiation.

Table 3. The level of DNA damage in yeast cells under the action of various doses of X-ray radiation (dose rate of 4.5 Gy / min).

X-ray dose, Gy	Comet tail length, μm	DNA content in the tail of a comet, %
Control	0.052 ± 0.019	0.16 ± 0.08
2	0.060 ± 0.021	0.17 ± 0.07
5	0.080 ± 0.020	0.39 ± 0.11
10	0.098 ± 0.014*	0.53 ± 0.09**
20	0.118 ± 0.015*	0.76 ± 0.25**
40	0.215 ± 0.029*	2.62 ± 0.75**
80	0.400 ± 0.045*	6.79 ± 1.75**

*p < 0,05.
**p < 0,02 – significant differences from the control by Student t-test.

Table 4. The level of DNA damage in peripheral blood leukocytes of mice under the influence of various doses of X-ray radiation (dose rate 1 Gy / min).

X-ray dose, Gy	Comet tail length, μm	DNA content in the tail of a comet, %
Control	0.31 ± 0.20	0.029 ± 0.019
0.1	0.28 ± 0.28*	0.028 ± 0.028*
0.2	3.54 ± 1.43*	0.50 ± 0.20*
0.5	4.77 ± 1.48*	0.71 ± 0.20*
1.0	10.1 ± 3.1*	1.71 ± 0.60*
2.0	19.9 ± 3.0*	4.48 ± 0.86*
5.0	32.0 ± 5.7*	10.5 ± 2.1*
10.0	45.3 ± 7.1*	19.9 ± 3.7*

*p < 0,01– significant differences from the control by Student t-test.

Comparative analysis shows that the DNA content in the comet tail is about 2 % after irradiation of yeast spheroplasts at a dose of 40 Gy, whereas in peripheral blood leukocytes of mice, the DNA content in the comet tail reaches 2 % after irradiation at a dose of 1 Gy, which is approximately 40 times lower.

Thus, the comet test allows recording a significant increase in the level of DNA damage in yeast spheroplasts after X-ray radiation and MMS treatment. In this case, the dependence on the dose of radiation is almost linear.

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