Letitsia Oprean, Victor Nederitsa

USE OF YEASTS AS A BIOINDICATOR FOR THE ULTRAVIOLET RADIATION ACTION ON LIVING ORGANISMS

Lucian Blaga University of Sibiu, Faculty of Textiles and Food Processing,
7-9 Dr. Ratiu St., Sibiu, Romania

The microbial cell, having a light weight, is strongly influenced by the environmental conditions and reacts very quickly at different factors, either by adapting or, on the contrary, by disappearance. Thus, the microbial growth depends on numerous physical, chemical and biological factors, these leading to specific adaptations during their evolution, by interactions between micro-organisms and environment. One of the exogene factors of the natural/industrial environment that influences the microorganism is the radiant energy [1, 3].

The living world is bombarded with electromagnetic radiation with the wavelength between 10^{-4} and 10^{-7}. Within this range, the microorganisms are influenced by radiation in a specific way. Although certain wavelengths of the visible light are beneficial for some bacteria, as the photosynthesizing forms, usually solar light is detrimental to the most microorganisms. This effect is due mainly to the ultraviolet (UV) range of the spectrum, especially to this radiation with the wavelength from 260 and 254 nm, that have a lethal or mutagene effect on the living cell [4].

The ultraviolet radiation, depending on the dose and the microorganism status, has a maximal lethal effect at λ = 254 nm and cause the tryptophan degradation with forming of toxic compounds that led to the physiological dead of the exposed cell. If the dose is sublethal, the radiation induces modifications in the DNA structure, favoring the thymine molecules coupling; the genetic information is transmitted with errors and mutants can be obtained.

The microorganisms irradiated with a lethal dose of UV radiation can not replicate and die. The microbicidal effect of the culture exposure to the light with UV radiation can be reduced by immediate exposure to visible light, with the λ between 365 and 450 nm, when the microorganisms can rebuild their initial structure. This action, opposite to the killing one, is called photoreactivation. By photoreactivation, the visible light activates the enzymes that separate the thymine dimers formed after the UV irradiation or in darkness, when the cell has the capability to eliminate the denaturated portion. Thus, the number of the surviving (living) cells of a microbial population that underwent the action of the visible light after UV irradiation is greater than in case of the populations treated only with UV radiation. In practical terms, the UV radiation can be used for air sterilization and for obtaining valuable mutants, used because of their biosynthesis products [5].

The paper describes comparative researches on the fungicidal (lethal) effect of the exposure of a Saccharomyces cerevisiae baker's yeast culture to UV radiation (λ = 254 nm) and the reduction of this effect by photoreactivation with visible light of a yeast culture irradiated with UV radiation.

The yeasts are ideal for these research activities because of many reasons:

– the yeasts grow in colonies that can be observed within 24 hours, at 30°C (by incubation in thermostat) or at room temperature, after 3–4 days;

– the yeast cells are eukaryotic ones and are more similar to the human cells than bacteria;

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– the UV light is used for these researches because it is known that it provokes genetic destruction at the DNA molecule level;

– due to the destruction of the ozone layer, the problems caused by the ultraviolet radiation on human and animal skin is a sensitive topic.

Materials and methods

As study material it was used a young (24 hours) Saccharomyces cerevisiae baker's yeast culture, and as UV radiation source it has been used laboratory UV microbicidal (sterilisated) lamp (UV radiation, with $\lambda = 254$ nm).

To assess the action that IR radiation has on the morphology and physiology of the Saccharomyces cerevisiae baker's yeast cells, there were performed the following determinations:

1. In the samples treated with IR radiation it was determined the total number of living cells per/1mL (TNG), by using the Koch culture method and it was calculated the survival percentage (% of the living irradiated cells).
2. For the morphological and physiological characterization of the non-irradiated and irradiated yeast cells, there were made the following analyses:

– Visualization of the form and the determination of these cells dimensions, as well as the identification of their cellular structure (buds, cell wall, cell membranes, nucleus, vacuoles, glycogen inclusions and spores). These were made by microscopic investigation on wet smears, where the yeast cells were suspended in Lugol solution. The Lugol solution enters the cell and colors the glycogen inclusions in reddish-brown. The presence of these intra-cellular inclusions in great amount certifies the active state of the yeast cells.

– Visualization of the autolysed cells: a cell suspension was mixed in equal ratio with a diluted methyl blue (containing methyl blue 0.01% m/m and natrium citrate · 2 H_2O, 2 g) and after 3–5 minutes it was made a wet smear. In the smear, the autolysed cells appear intensively colored in blue, compared to the living cell, colorless. Methyl blue protrudes in both the living cells and the autolysed ones, but in the living ones are active the reductases, enzymes that reduce the redox indicator to a colorless leuco-derivative, therefore the living cells appearing colorless in the smear. In the autolysed cells the reductases are inactivated (by the action of the hydrolytic enzymes, active at autolysis), therefore the reduction reaction does not take place and in the wet smear the autolysed cells are colored in blue.

– Visualization of the ascospores presence in the irradiated yeast cells. It was used the method of coloring them with phenycated fuchsin. At microscope the spores appear colored in red.

The working technique used for the irradiation with UV radiations and for the photoreactivation of the Saccharomyces cerevisiae yeast cells has consisted in the following steps:

1. From the 10^{-5}, 10^{-6} and 10^{-7} dilutions, obtained from a pure culture of Saccharomyces cerevisiae, there have been made inoculations on a malt-agar extract medium in Petri dishes, using the Koch method [2, 6]. To obtain the most exact results, from the same dilution there have been made inoculations on two parallel dishes. After 72 hours (3 days) of incubation in thermostat, at 30°C, the yeast colonies formed on these dishes were counted and the total number of living yeast cells in 1 mL (TGN) was determined (control samples) [2, 6].

2. From the control 10^{-5} dilution are poured 2 mL in 4 sterile Petri dishes and on each plate it is noted the irradiation time: 5 sec., 10 sec., 15 sec., 20 sec.

3. The Petri dishes are arranged in the increase order of the irradiation time inside the exposure box. The plates are turned and then exposed to UV radiation, covered with aluminium foil, to prevent the light entrance.

4. Right after the UV radiation exposure it is prepared the 10^{-6} dilution of each irradiated sample, by transferring 0.1 mL of liquid from each plate to sterile ones and it is poured malt-agar extract, fluidized and cooled at 45°C; after gentle stirring, the plates are covered with aluminium foil. After 72 hours of incubation in thermostat, at 30°C, it is determined the total number of living yeast cells/mL (TGN).

5. From each Petri dish irradiated with UV radiation it is transferred 1 mL in a sterile test-tube, on which the UV irradiation time is noted.

6. These test-tubes are treated with visible light from a 500 W light bulb, for 30 min., placed into a vessel with ice, in order to prevent heating.

7. Right after the visible light exposure it is prepared the 10^{-6} dilution of each photoreactivated sample, by transferring 0.1 mL of liquid from each photoreactivated test-tube, on sterile plates and it is poured malt-agar

extract, fluidized and cooled at 45°C; after gentle stirring, the plates are covered with aluminium foil. After 72 hours of incubation in thermostat, at 30°C, it is determined the total number of living yeast cells/mL (TGN).

8. In the UV irradiated samples and in the photoreactivated ones it is calculated the survival percentage (irradiated and photoreactivated living cells).

Results and discussions

After 3 days of incubation at 30°C on agar malt extract, the Saccharomyces cerevisiae baker's yeast forms S-type colonies, with 1–2 mm in diameter, with round perimeter, sometimes triangle lobbed, convex shape, creamish consistency, smooth and shiny aspect, colored in creamish-white. After 5 days, the colonies reach 2.5–5 mm in diameter and maintain the morphological characteristics.

In the liquid medium (malt extract) they generate turbidity and on the surface they form a fine foam, with mat aspect. At the fermentation end, after 2 hours they form a compact sediment.

Microscopically, after 3 days, the cells have spherical or oval shape, with medium dimensions (3.5–10) x (5–12) μm, some being ellipsoidal prolonged, other rounded. They are disposed isolated, in pairs or chains. There were also seen cells with buds of different sizes, arranged on poles. The cells content is transparent, with fine foamy aspect, having white-grayish reflexes and it is rich in vacuoles. In the wet smear, prepared from a droplet of yeast and one of Lugol solution it was noted the presence of a large number of glycogen inclusions, these being colored in reddish-brown, thus proving the active state of the yeast cells.

The results obtained in studying the fungicidic effect of the exposure of a Saccharomyces cerevisiae baker's yeast culture to UV lethal radiation and the reduction of this effect by photoreactivation with visible light of a baker's yeast culture irradiated with UV radiation are presented in Table.

The control culture of Saccharomyces cerevisiae contains 175 x 10^6 cells/mL.

Action of the UV radiation and photoreactivation on the Saccharomyces cerevisiae baker's yeast cells

Irradiation time	Irradiated yeast cells		Photoreactivated yeast cells	
(seconds)	Number of living cells x 10^6	Percentage of living cells	Number of living cells x 10^6	Percentage of living cells
5	142	81.14	161	92.00
10	98	56.00	136	77.71
15	37	21.16	91	52.00
20	10	5.71	36	25.14

As far as the toxicity of the UV radiation on the Saccharomyces cerevisiae yeast cells, it can be seen in table 1 that this radiation have a different fungicidal effect, depending on the exposure time. The minimal and maximal fungicidal effect of the UV radiation on Saccharomyces cerevisiae was noted after 5 seconds and 20 seconds of irradiation, respectively (after 20 seconds there remains no living cells).

The Saccharomyces cerevisiae culture exposed to UV radiation for 5 sec. has a high content of living irradiated cells, the value being with 24.57% smaller that in control (not irradiated sample).

In the Saccharomyces cerevisiae cultures, exposed to UV radiation for 10 and 15 sec., it can be seen a decrease of the living irradiated cells, the values being with 44.00% and 78.48% smaller than in control, respectively.

In the Saccharomyces cerevisiae cultures, exposed to UV radiation for 20 sec., it can be seen a decrease of the living irradiated cells, the values being with 94.29% smaller than in control (not irradied sample).

The Saccharomyces cerevisiae baker's yeast cells, exposed to infrared radiation for 5, 10, 15 and 20 sec. and examined at microscope, on wet smears, have different cellular, cultural and physiological characters, depending on the irradiation time; thus there were noted different states of the irradiated yeast cells:

1. Living yeast cells, intact, without modifications, separated ones from the others. In shape they are identical with the yeast cell from the blank culture, but their dimensions are smaller. The cytoplasm is granulated, with smaller and fewer vacuoles. On wet smears colored with Lugol solution, the glycogen inclusions are colored in reddish-brown, their number being smaller than that in the blank culture. They form identical colonies as the non-irradiated cells (blank).

2. Slightly affected living cells, but which can re-take the normal functions. These present slight morphological damages, reversible, with the thickening of the yeast cell, that acquires an irregular contour. In this "resistance" stage, the integrity of the yeast cell is preserved. They form colonies that have the same shape that the intact cells ones, but with smaller dimension.

3. Cells with more accentuated and hardly reversible modifications, with more accentuated thickening of membrane and cell wall, with protoplasm clarification, together its more or less complete disintegration, but preserving the integrity of the cell wall. The cells form is irregular, wrinkled and the vacuoles are very diminished. On smears colored with diluted methyl blue solution, in the majority of the observed cells, the cell content look colored in blue, the membrane and cell wall being less or not at all colored, this indicating that the cells are dead. They form colonies that have the same form as the intact cells ones, but less developed and with much smaller dimensions.

At microscope there were also visualized sporulated yeast cells. On wet smears the spores can be seen without special coloring. Thus, in the cell interior, instead of cytoplasm there were observed 2–4 ascospores. To render them more evident, they were colored with phenycated fuchsin solution, the spores appearing colored in red.

4. Dead (autolysed) cells. These cells have serious and irreversible morphological damages, with advanced cytolysis, membrane destruction and fragmentation of the cell wall. Observed at microscope, in wet smears colored with diluted solution of methyl blue, the cells appear with some dispersed grains and fat droplets, that strongly refract light and are colored in intense blue. Being dead cells, they do not form colonies on the agar must extract.

The number of living cells of a photereactivated culture of Saccharomyces cerevisiae is, as seen in table 1, greater than in the UV irradiated culture.

In the Saccharomyces cerevisiae culture UV irradiated for 5 sec. and photoreactivated it is noted an increase of the living cells amount, the value being with 16.57% greater than in the UV irradiated sample and with 8% smaller than in control (not irradied sample).

In the Saccharomyces cerevisiae cultures exposed to UV for 10 and 15 sec. and photoreactivated the number of living cells is also increased, with 25.22% and 8.11% greater than those exposed only to UV radiation, respectively and with 22.29% and 48.00% smaller than in control, respectively.

In the Saccharomyces cerevisiae culture exposed to UV for 20 sec. and photoreactivated the number of living cells is with 14.86% greater than in case of exposure to UV radiation only and with 74.86% smaller than in control.

Conclusions

1. The ultraviolet radiation has a differentiated fungicidal (lethal) effect on the Saccharomyces cerevisiae baker's yeast cells, according to the yeast cells initial concentration an irradiation time. By the increase order of the expose time of the yeast cells to the UV raddiations, the rate of survival of the irradied living cells descreases.
2. The changes of the yeasts under the action of UV radiations are variable and concern the shape, dimensions, celulare structure and the type of the culture. Under the intensity of these actions on the yeast cells morfological, slight, reversible damages, have occured important, hard reversible changes, and serious, irreversible damages, with increased autolise have occured. Remarcable, is the production of ascosporus in the irradiated yeast cells.
3. The number of living cells in the Saccharomyces cerevisiae culture that was exposed to the visible light action after UV irradiation (photoreactivation) is greater than that in the yeast culture exposed only to UV radiation.
4. Thus, there is possible to use the Saccharomyces cerevisiae yeast as a simple and efficient bioindicator of the UV radiation effect on living organisms.

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Summary

The paper presents the results of the comparative research on the fungicidal (lethal) effect of the exposure of a Saccharomyces cerevisiae baker's yeast culture to UV radiation and the reduction of this effect by photoreactivation with visible light of the UV irradiated culture. For the cultivation of control and irradiated yeasts as well as for the numbering of the living cells/mL (TGN), there have been used the standard methods. The ultraviolet radiation has a differentiated fungicidal effect on Saccharomyces cerevisiae baker's yeast cells, depending on the initial concentration of the yeast cells and the irradiation time. The changes of the yeasts under the action of UV radiations are variable and concern the shape, dimensions, celulare structure and the type of the culture. Thus, there is possible to use the Saccharomyces cerevisiae yeast as a simple and efficient bioindicator of the UV radiation effect on living organisms.