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RESEARCHES ON THE ACTION OF THE ULTRAVIOLET RADIATION ON BACTERIA

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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 microorganisms 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 10^6. 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 bacteria. 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 [3, 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 thiamin molecules coupling; the genetic information is transmitted with errors and mutants can be obtained. The irradiated bacterial cell thus cannot reproduce and dies.

The bactericidal 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 thiamin dimmers formed after the UV irradiation or in darkness, when the cell has the capability to eliminate the denatured 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.

The papers describes comparative researches on the bactericide (lethal) effect of the exposure of a Escherichia coli culture to UV radiation (λ = 254 nm) and the reduction of this effect by photoreactivation with visible light of a bacterial culture irradiated with UV radiation [5].

Materials and method

As study material it was used a young (24 hours) Escherichia coli bacterial culture, and as UV radiation source it has been used laboratory UV microbicidal (sterilisated) lamp (UV radiation, with λ = 254 nm). The working technique has consisted in the following steps:

1. From the 10^{-6}, 10^{-7} and 10^{-8} dilutions, obtained from a pure bacterial culture of Escherichia coli,

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there have been made inoculations on a lactose agar broth medium in Petri plates, using the Koch method [6]. To obtain the most exact results, from the same dilution there have been made inoculations on two parallel plates. After 72 hours (3 days) of incubation in thermostat, at 30°C, the bacterial colonies formed on these plates were counted and the total number of living bacterial cells in a ml (TGN) was determined (control samples) [6].

2. From the control 10^{-6} dilution are poured 2 ml in 4 sterile Petri plates and on each plate it is noted the irradiation time: 5 sec., 10 sec., 15 sec., 20 sec.

3. The Petri plates are arranged in the increase order of the irradiation time inside the exposure box. Remove all the Petri dish lids and turn on the UV light. Replace the lids and remove the plates at the appropriate 5-second intervals. Wrap with aluminum foil to prevent possible photoreactivation.

4. Right after the UV radiation exposure it is prepared the 10^{-7} dilution of each irradiated sample, by transferring 0.1 ml of liquid from each plate to sterile ones and it is poured lactose agar broth, fluidized and cooled at 45°C; after gentle stirring, the plates are covered with aluminum foil. After 72 hours of incubation in thermostat, at 30°C, it is determined the total number of living bacterial 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^{-7} dilution of each photoreactivated sample, by transferring 0.1 ml of liquid from each photoreactivated test-tube, on sterile plates and it is poured lactose agar broth, fluidized and cooled at 45°C; after gentle stirring, the plates are covered with aluminum foil. After 72 hours of incubation in thermostat, at 30°C, it is determined the total number of living bacterial 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

The results obtained in studying the bactericide (lethal) effect of the exposure of a Escherichia coli bacterial culture to UV lethal radiation and the reduction of this effect by photoreactivation with visible light of a bacterial culture irradiated with UV radiation are presented in Table.

Action of the UV radiation and photoreactivation on the Escherichia coli bacterial cells

Irradiation time	Irradiated bacterial cells		Photoreactivated Bacterial cells	
(seconds)	Number of living cells x 10^7	Percentage of living cells	Number of living cells x 10^7	Percentage of living cells
5	131	73.07	156	85.43
10	85	46.71	115	63.18
15	26	14.28	62	34.06
20	8	4.39	41	22.52

The survival curve, expressing the relationship between the number of living bacterial cells after irradiation/photoreactivation vs. the irradiation time is presented in figure 1.

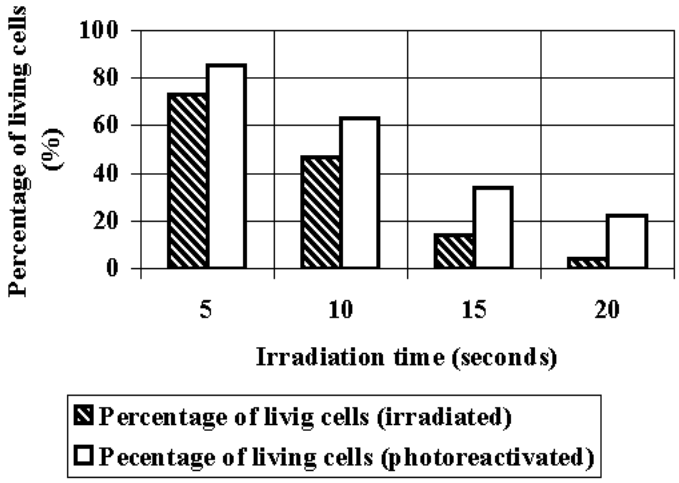
The control bacterial culture of Escherichia coli non irradiated contains 182 x 10^7 cells/ml.

As far as the toxicity of the UV radiation on the Escherichia coli bacterial cells, it can be seen in table and fig. that this radiation have a different bactericide effect, depending on the exposure time. The minimal and maximal bactericide effect of the UV radiation on Escherichia coli was noted after 5 seconds and 20 seconds of irradiation.

The Escherichia coli bacterial culture exposed to UV radiation for 5 sec., it can be seen a decrease of the living irradiated cells, the value being with 26.93% smaller that in controls (not irradiated sample).

In the Escherichia coli bacterial 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 53.29% and 85.72 % smaller than in control, respectively. The Escherichia coli bacterial culture exposed to UV radiation for 20 sec., it can be seen a decrease of the living irradiated cells, the value being with 95.61% smaller that in control. The number of

living cells of a photereactivated culture of Escherichia coli is, as seen in Table and Fig. greater than in the UV irradiated culture.



The rate of living bacterial cells after irradiation/photoreactivation depending on the irradiation time.

In the Escherichia coli bacterial culture UV irradiated for 5 sec. and photoreactivated the number of living cells is also increased, the value being with 12.36% greater than in the UV irradiated sample.

In the Escherichia coli bacterial cultures exposed to UV for 10 and 15 sec. and photoreactivated the number of living cells is also increased, the value being with 16.47% and 19.78% greater than in the UV irradiated sample.

In the Escherichia coli bacterial culture UV irradiated for 20 sec. and photoreactivated the number of living cells is also increased, the value being with 18.13% greater than in the UV irradiated sample.

Conclusions

1. The ultraviolet radiation has a differentiated bactericide (lethal) effect on the Escherichia coli bacterial cells, according to the bacterial cells initial concentration an irradiation time.
2. The number of living cells in the Escherichia coli bacterial culture that was exposed to the visible light action after UV irradiation (photoreactivation) is greater than that in the bacterial culture exposed only to UV radiation.

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Summary

The paper presents the results of the comparative research on the bactericid (lethal) effect of the exposure of a Escherichia coli bacterial culture to lethal UV radiation and the reduction of this effect by photoreactivation with visible light of the UV irradiated culture. As UV radiation source it has been used laboratory UV microbicidal (sterilisated) lamp (UV radiation, $\lambda = 254$ nm). For the cultivation of control and irradiated bacterial culture of E. coli 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 bactericide effect on Escherichia coli cells, depending on the initial concentration of the bacterial cells and the irradiation time. The number of living cells in the E. coli bacterial culture that was exposed to the visible light action after UV irradiation (photoreactivation) is greater than that in the bacterial culture exposed only to UV radiation.