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STUDY OF THE MORPHO - CULTURAL CHARACTERISTICS OF SOME MICROORGANISMS IRRADIATED WITH IONIZING GAMMA RADIATION

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Abstract. The article is devoted to the study of morpho - cultural changes that occur as a result of the impact of different doses of ionizing gamma radiation on microorganisms, which is one of the stress factors. After irradiation, preparations made from the studied microorganisms (yeast fungus and lactic acid bacteria) were studied under a light microscope. During the observation of morpho - cultural characteristics, it was observed that the size of the colonies was smaller in the samples irradiated with a high dose (3000 Gy), and in contrast to the size of the colonies in the small doses (500 Gy), it was observed that it was larger. It was also determined that bacteria have similar morphophysiological characteristics - spherical (coccal), immobile form. Morphologically, it was known that sample cells irradiated with a high dose were closer to each other under the microscope.

Keywords: *ionizing gamma radiation, yeast, lactic acid bacteria, stress factors, morpho-cultural parameters.*

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1. Introduction

There are many scientific studies on the effect of ionizing gamma radiation on biological systems (Nasibova & Khalilov, 2016; Nasibova, 2020; Khalilov *et al*., 2011; Kavetskyy *et al*., 2018). Studies on the interaction of radiation with biological systems have benefited society in medical biology, agriculture, pharmaceuticals and other technological processes (Khoeini *et al*., 2021; Maleiki Dizaj *et al*., 2021). The determination of the inhibitory dose of radiation to microorganisms, especially in the fields of agricultural sciences and food technology, revealed the potential application of radiation in the field of microbial disinfection.

In many scientific studies, lethal, semi-lethal, sublethal or stimulating doses of ionizing gamma radiation to plant and animal organisms have been determined (Khalilov and Nasibova, 2008, Khalilov *et al.*, 2018; Nasibova, 2019). Also, in recent decades, scientists have been particularly interested in gamma rays for the evolution of superior varieties of economically important agricultural plants. Many general information, such as the effect of ionizing gamma radiation on plants, lethal dose, beneficial properties, etc., have been studied by observing growth and morphological changes (Jan *et al.*, 2012).

In previous studies, different types of plants exposed to different stress factors were studied. Morphological features of wheat (*Triticum*) of C3 type of photosynthesis and corn (*Zea mays*) of C4 type of photosynthesis plants were studied under the

influence of radiation factors, the mechanism of the obtained results was clarified and at the same time paramagnetic centers were studied in them (Borzouei *et al.*, 2010).

When we studied the effect of radiation on the morphology of cotton (*Gossypium L.*) plant samples in our research studies, we found that a low dose of ionizing gamma radiation (60 Gy) has a stimulating effect on the growth and development of the cotton plant, and a high dose (200 Gy) has a retarding effect (Kazımlı, 2018). The influence of radioactive radiation on pomegranate (*Punica granatum*), fig (*Ficus carica L.*), eldar pine (*Pinus eldarica M.*) and other plants was studied, and for the first time, the emergence of new magnetic properties in them under the influence of stress factors was determined (Nasibova *et al*., 2016, 2021(a), 2021(b), Ahmedov *et al*., 2020).

The obtained results were also confirmed in experiments with some animal organisms (Khalilov *et al*., 2008; Nasibova *et al*., 2021; Maleki Dizaj *et al*., 2021).

The main goal of the presented work is to study the change of morpho - cultural characteristics depending on the dose in the yeast strain and lactic acid bacteria exposed to different doses of ionizing gamma radiation.

2. Materials and methods

In the article, the morpho - cultural changes that occurred as a result of the impact of ionizing gamma radiation, which is one of the stress factors, on some microorganisms with different doses were studied. Microorganisms used in the research were yeast strain Candida guilliermondii BDU-217 and lactic acid bacteria. Lactic acid bacteria strains were obtained from yogurt and cheese samples from different agro-climatic zones of Azerbaijan. 8 strains were isolated from yogurt samples and 5 from cheese.

If we look at the biochemical and microbiological characteristics of glandular lactic acid bacteria (LAB), they are a group of bacteria functionally similar in their ability to produce lactic acid by homo- or heterofermentative metabolism. They are non-pathogenic, gram-positive, non-spore-forming, facultative anaerobes with high 12 enzymatic activity in terms of oxygen. During development, they acidify the environment quite strongly. This group is morphologically heterogeneous. LAB belongs to 3 families: *Lactobacteriaceae*, *Streptococcaceae* and *Peptococcaceae*. Typical LAB species belong to the *Streptococcus*, *Lactobacillus* and *Leuconostoc*. LAB can only ferment, use carbohydrates as an energy source and secrete lactic acid. Bacteria of this group are catalase-negative, do not contain cytochromes and catalase-like hemoproteins. But despite this, they are able to grow in the presence of air oxygen: for being anaerobic, they are still aerotolerant.

It should be noted that yeast fungi are one of the widely used model objects in scientific research and practice since ancient times. Yeast fungi have many unique valuable properties, one of which is tolerance to stress factors (temperature, UV-rays, gamma radiation, etc.), easy separation from nature, optimal size and high reproduction rate. Yeast fungi are very common in nature. These do not form endospores. Very few species have mycelia. The cells are oval, elongated and stalk-like, they occur singly or in chains. It is pigment free. Many species have developed mycelia. Later, the filaments divide into short yeast-like cells. Some of these retain their yeast-like appearance, while others remain in mycelial form. These mushrooms are little studied and their classification is done only based on their morphological and physiological characteristics.

Lactic acid bacteria are used in the dairy and food industry, agriculture and households, as well as in medicine, due to their sterilizing and protective effect. Fermentation of various foods with lactic acid bacteria is one of the ancient biological safety methods used by humans. LAB is used as a natural and selective starter for food fermentation, where the fermentation process is carried out due to the production of lactic and acetic acids (Egorov *et al*., 1999; Karaev *et al*., 2006; Cadirci *et al*., 2015). They are auxiliary products to improve hygienic safety, storage stability and palatability of products. Lactic acid bacteria protect food from spoilage and pathogenic microorganisms by producing organic acids, hydrogen peroxide, diacetyl, antifungal components and bacteriocins. In the production of hard cheeses, renal casein is used for freezing and lactic acid bacteria in the ripening stage of cheeses. Yogurt is obtained from pasteurized homogenized whole milk inoculated with *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Axelsson *et al*., 2013; Gulahmadov *et al*., 2007; Tsakalidou *et al*., 1993; Zsolt *et al*., 2015). Pure lactic acid, which is used for various industrial purposes and as an additive to food products, is obtained by fermenting milk using *L. casei* or *L. bulgaricus*. Acid formation in the dough of flour used in the preparation of dough is carried out by lactic acid bacteria, especially *L. plantarum* and *L. coryneformis*. Starter cultures of lactobacilli and micrococci are also used to make uncooked smoked sausages. Lactic acid bacteria protect uncooked sausages from spoilage by producing acid and lowering the pH. Lactic acid bacteria are widely used in the brewing industry. Fermented dairy products have great economic value and play an important role in human health.

Considering that the radiation background existing on the earth is less than in the early times, but still our planet is under the influence of the radiation background. It is for this reason that yeast fungi from living systems have attracted the attention of scientists with their persistence in this field. In general, it has been known that these fungi have high radioresistance when exposed to high doses of gamma radiation (Dadachova *et al*., 2008). Effects of ionizing radiation on some living systems, including paramagnetic centers, have been studied by Electron Paramagnetic Resonance spectroscopy (EPR) and other methods (Nasibova *et al*., 2017). However, studies on the effect of gamma radiation on the change of morphocultural characteristics in microorganisms are still limited (Kazimli, 2022; Gunashova & Khalilov, 2022).

The yeast strain was first incubated in the malt - agar nutrient medium for 3 days in a thermostat at 30˚C and kept in a refrigerator for further research. This strain was irradiated in the MRH gamma-25 device at doses of 500 Gy, 1500 Gy and 3000 Gy in laboratory conditions with one control (Fig. 1).

In order to study the morpho - cultural characteristics of the samples after irradiation, after sterilizing the bacterial ring, it was taken from the biomass and spread on a glass slide. In order to obtain an image of the cells under a microscope, a preparation was prepared from the samples using funxin colorant, and observations were made under an XSP - 30 microscope.

The XSP-30 microscope is suitable for use in high schools, universities, colleges, medical and healthcare institutions, clinical monitoring and various fields. It has the ability to zoom up to 40x-1600x.

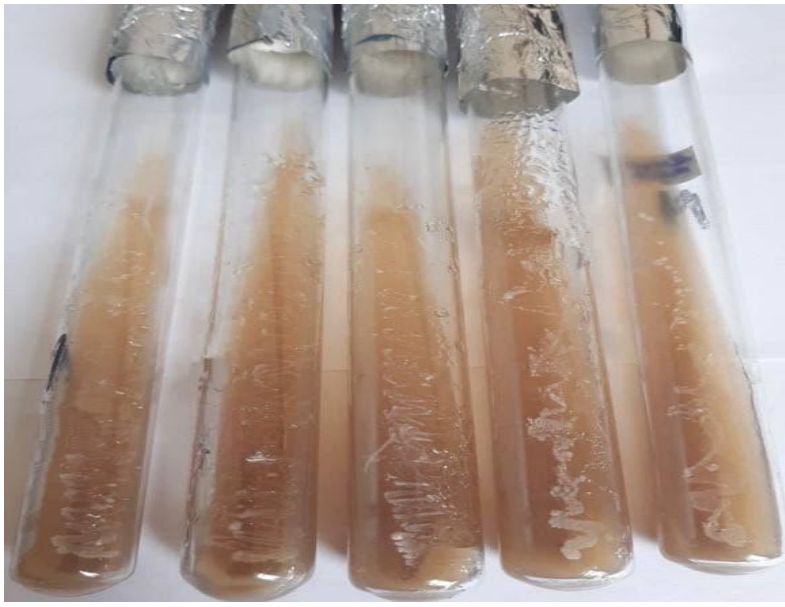
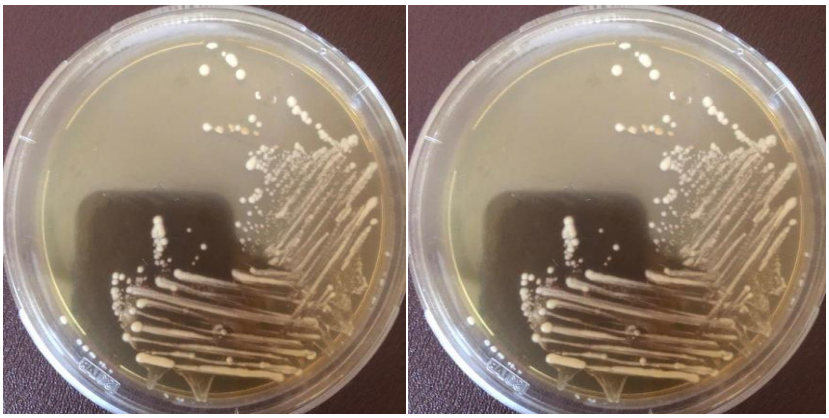


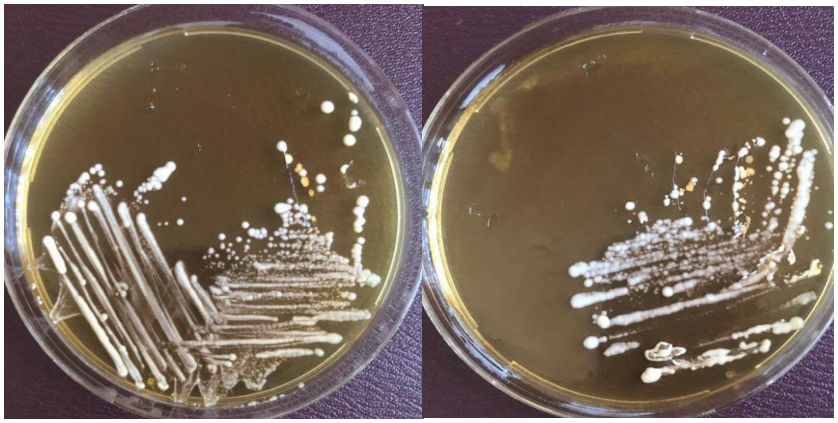
Fig. 1. Malt-agar nutrient medium, in test bottles
cultivated yeast strain Candida guilliermondii BDU-217

3. Result and discussion

In our experiments, 2 types of milk products were used. Group 1 samples were isolated from yogurt and group 2 samples from cheese. These products were obtained from different areas of Azerbaijan in order to isolate wild type lactic acid bacteria (LAB) from products prepared under natural conditions without the participation of biopreservatives. Screening of LAB with antimicrobial activity was performed among cocci-like bacterial strains. The yogurt starter is made with cow's milk without the addition of microorganisms. Colonies isolated from yogurt are numbered and marked with the letter Y, and colonies isolated from cheese samples are conventionally marked with the letter C (Fig. 2).



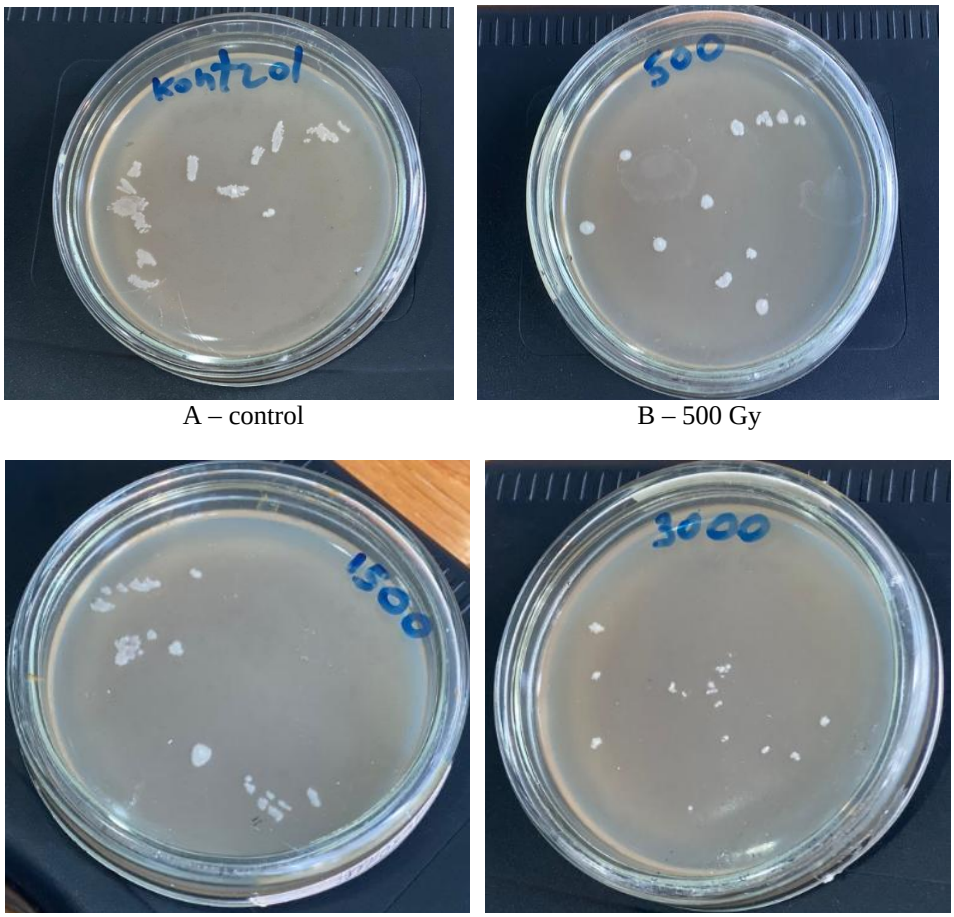
A (Y) B (C)



C (C) D (C)

Fig. 2. Isolation of LAB from yogurt a (Y) and cheese b,c,d (C) samples

In order to determine the effect of ionizing radiation on morphological changes, bacterial samples were irradiated at different doses - 200 Gy, 400 Gy, 600 Gy, 800 Gy and 1000 Gy in the MRH gamma - 25 device, and their morpho-cultural characteristics were studied.



A – control B – 500 Gy

C – 1500 Gy D – 3000 Gy

Fig. 3. A-control and B-500 Gy, C-1500 Gy, D- 3000 Gy doses of Candida guilliermondii BDU-217 yeast strain planted in Petri dishes

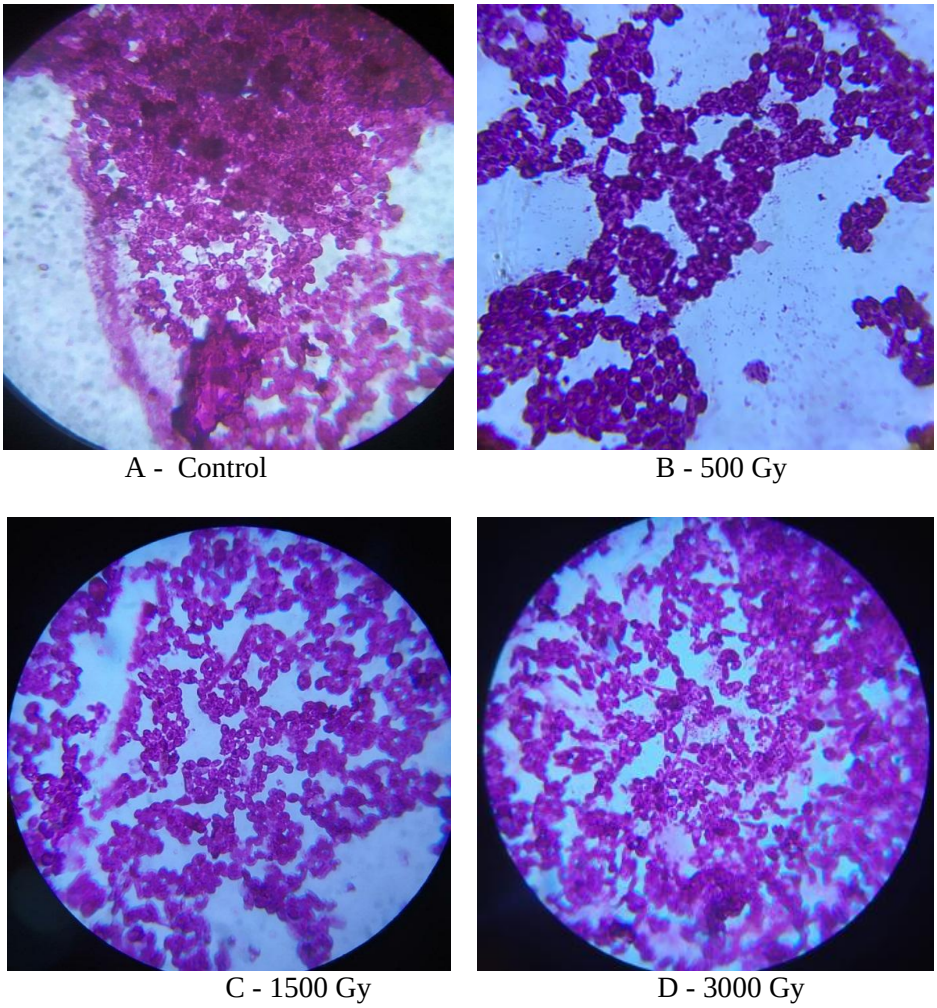


Fig. 4. A-control and B-500 Gy, C-1500 Gy, D- 3000 Gy irradiated Candida guilliermondii BDU-217 yeast strain under a microscope image

In our study, we observed a number of morphological changes in Candida guilliermondii BDU-217 yeast strain and lactic acid bacteria under the influence of different doses of gamma radiation. During the observation of the morphological image of the samples in petri dishes after irradiation, it was observed that the size of the colonies was smaller in the sample irradiated with a higher dose, and the size of the colonies was larger in the case of lower doses (Fig. 3).

At the next stage of the research, preparations were made from the samples and observations were made under a microscope. At this time, it was known that sample cells irradiated with a higher dose were closer to each other under the microscope. This is the explanation of the response of cells to stress (Fig. 4).

The same observations were made in lactic acid bacteria. As can be seen from table 1, the dose increase caused a decrease in the size of colonies in the morphological appearance of lactic acid bacteria as well as in yeast.

Table 1. Sensitivity of lactic acid bacteria to radiation

Radiation dose, (Gy)	Number of colonies visualized (LogCFU/ml)
control	7.1
200	6.8
400	6.5
600	5.8
800	5.2
1000	5

4. Conclusion

In our research, we observed a number of morphological changes in Candida guilliermondii BDU-217 yeast strain and lactic acid bacteria under the influence of different doses of gamma radiation. During the observation of the morphological image of the samples in petri dishes after irradiation, it was observed that the size of the colonies was smaller in the sample irradiated with a higher dose, and the size of the colonies was larger in the case of lower doses. In addition, morphologically, it was known that sample cells irradiated with a high dose were closer to each other under the microscope.

The morpho - cultural characteristics of the studied microorganisms were studied under the influence of different doses of ionizing gamma radiation. In our ongoing research, the study of paramagnetic centers in these microorganisms under the influence of stress factors is carried out, which is of great importance in biomedical and ecological research.

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