GAMMA RADIATION EXPOSURE OF SOME BIOTECHNOLOGICAL BACTERIAL STRAINS

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Abstract. The present study aimed to evaluate the effect of gamma radiation on the development of two strains of *Pseudomonas* sp. as well as the stimulatory effect of various gamma radiation doses on biologically active substances of biotechnological interest. The tests performed have shown that small irradiation doses, of 10-50 Gy, do not influence the growth rate, as the lethal dose for both *Pseudomonas* C16 and respectively *Pseudomonas aeruginosa* 3d strains is 1000 Gy. Doses of 100, 200 Gy improve the quality of surfactants; gamma irradiation also affects the synthesis of bacterial products, resulting in a positive response of some bacterial strains to certain levels of irradiation.

Keywords: Gamma radiation, bacterial strains, bacterial surfactant, polyhydroxyalcanoats.

Rezumat. Expunerea unor tulpini bacteriene de interes biotehnologic la radiații gamma. Prezentul studiu a urmărit efectul radiaţiilor gama asupra dezvoltării a două tulpini de *Pseudomonas* sp., cât şi efectul stimulativ al diferitelor doze de radiații gamma asupra unor substanţe biologic active de interes biotehnologic. Testele întreprinse au demonstrat, că dozele mici de iradiere, 10-50 Gy nu influenţează rata de creştere, doza letală pentru tulpinile *Pseudomonas* C16 şi respectiv tulpina *Pseudomonas aeruginosa* 3d, este la valoarea de 1000 Gy. Doze de 100, 200 Gy imbunătăţesc calitatea surfactanţilor; de asemenea iradierea Gamma influențează sinteza de produşi bacterieni, obţinându-se un răspuns pozitiv al unor tulpini bacteriene la anumite nivele de iradiere.

Cuvinte cheie: radiație gamma, tulpini bacteriene, surfactanți bacterieni, polihidroxialcanoaţi.

INTRODUCTION

Gamma radiation is an electromagnetic radiation resulting from the radioactive disintegration of atomic nuclei. It was discovered in 1900 by the French chemist Paul Villard, while studying the radiation emitted by radium, the name of gamma rays being given in 1903 by Ernest Rutherford (GRASTY, 1976). Over time, gamma radiation has become useful in various fields, with a wide range of applicability in food industry, medicine, environment and conservation of patrimony objects.

Gamma radiations are used for the sterilisation of food and spices (anise, cumin, red pepper) the applied dosages reaching values of up to 10 kGy in red pepper powder (LEE et al., 2005). In the medical field, sterilisation by irradiation is an effective method of removing microorganisms (WHITE et al., 1994), but there are also radiation-resistant species of microorganisms as reported by Abe Anellis in 1973.

EZZAT et al. (2014) suggests gamma irradiation as an unconventional and effective method for treating waste water for pollution control. Another application area refers to some bacterial bioproducts influenced by the exposure of certain bacterial strains to gamma irradiation (BOURBRIK & ROUVIERE-ZANIV, 1995; ATIQUE et al., 2013), and the literature shows various studies related to the stimulative effect of gamma radiation on various bacterial metabolites.

Thus, the present study investigated the effect of gamma radiation on two bacterial strains belonging to the *Pseudomonas* genus, originating from the collection of the Institute of Biology, Bucharest, and exhibitingthe ability to synthesize bioproducts. The performed irradiation tests assessed the influence of gamma radiation on the growth rate and their stimulating effect on the bioproducts of interest, such as the polyhydroxyalkanoates and surfactants. It is noteworthy that the literature cites only a few studies on these metabolites.

MATERIAL AND METHODS

Two strains belonging to the *Pseudomonas* genus were used in the present study: one, isolated from soil samples, taken from the Copșa Mică industrial area contaminated with metal ions (*Pseudomonas* C16) and another one, *Pseudomonas aeruginosa* strain Ps3d, isolated from a sample taken from an area polluted with oil hydrocarbons. These strains have demonstrated the ability to synthesise polyhydoxyalkanoate and/or surfactant type products, products with a diverse range of use and a genuine biotechnological interest. Samples subjected to irradiation were cultivated on LB medium at a temperature of 28 °C, under constant stirring at 150 rpm/min.

The synthesis of the bioproducts of interest was highlighted by growing the selected and irradiated strains on selective media: for polyhydroxyalkanoates, a basal medium with sodium octanoateand Nile Red dye was used. The dye was introduced into the medium to a final concentration of 0.5 μg/ml, the colorimetric method allowing the identification of the intracellular accumulated PHA granules which appear fluorescent in UV light (312 nm) (SPIEKERMANN et al., 1999). The synthesis of biosurfactants was performed by culturing the samples on a mineral salt medium to which CTAB (cetyltrimethylammonium bromide) was added in a final concentration of 0.2g. (SIEGMUND & WAGNER, 1991; PINZON & JU, 2009). The oil dispersion test was also applied (PLAZA et al., 2006; NASR et al., 2009).

The samples, representing the 24-hour bacterial culture obtained on the LB medium at a temperature of 28 °C under stirring conditions at 150 rpm, were irradiated at various intensities, ranging from 10 to 2000 Gy in order to stimulate cell growth, but also metabolites production.

The irradiation was performed on volumes of 30 ml, in Falcon tubes, at the IRASM Technologic Irradiation Department of the „Horia Hulubei" Institute of Physics and Nuclear Engineering. A GC-5000, self-shielded, gamma source, consisting of 11 individual (pencil) sources, disposed in cylindrical geometry was used. For each dose point, 6 vials with biological samples were arranged in cylindrical geometry and placed symmetrically relative to the centre of the irradiation chamber and the Co-60 source.

Following irradiation, the cultures were re-incubated on LB medium, but also on specific media, the optical density of the inoculum being spectrophotometrically analysed. To this purpose, a UV/VIS spectrometer with a *FLUOstar Omega* multimode microplate readerwas used, the growth curves being recorded over 24 hours.

RESULTS AND DISCUSSIONS

Thepresent study aimed atdetermining the growth potential and the ability to synthesise bioproducts of interest (polyhydroxyalcanoates and biosurfactants) of *Pseudomonas* strains subjected to gamma irradiation.Cellular growth was assessed spectophotometrically, using a UV/VIS spectrometer with a FLUOstar Omega microplate reader, and by cultivation on a solid LB medium. As it can be seen in Figures 1 and 2, the development of both bacterial strains was inhibited by a 1000Gy radiation, which could be considered a lethal radiation, the value being within the reference range specified by Atique, 2013.

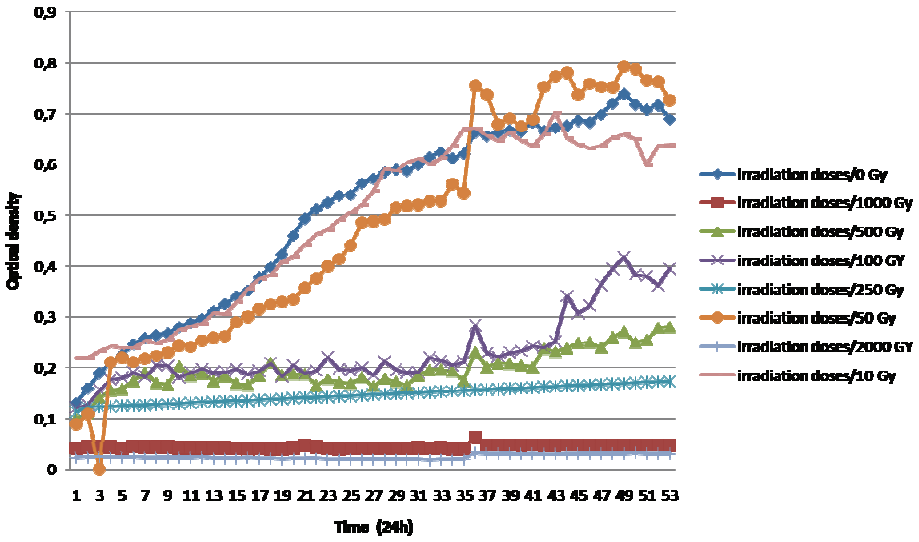

Figure 1. Development of *Pseudomonas aeruginosa* 3d strains after irradiation at different doses (10 Gy, 50 Gy, 100 Gy, 250 Gy, 500 Gy, 1000 Gy and 2000 Gy).

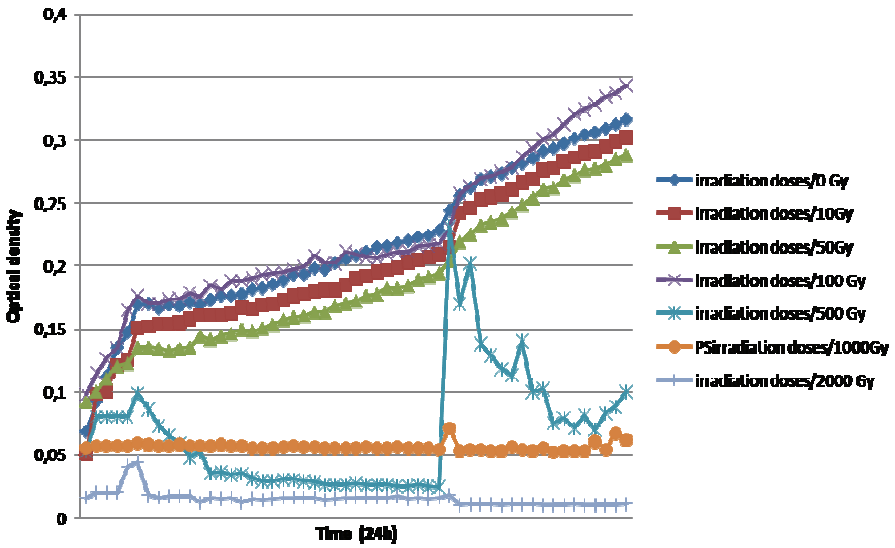

Figure 2. Development of *Pseudomonas* C16 strain after gamma irradiation at different doses (10 Gy, 50 Gy, 100 Gy, 250 Gy, 500 Gy, 1000 Gy and 2000 Gy).

There is also a decrease in cell growth, directly proportional to the increase in irradiation dose.Thus, at a 10 Gy irradiation, the development of bacterial culture is similar to that of the unirradiated control (0 Gy).

The spectrophotometric observations were also confirmed by cultivation of irradiated samples on agarised LB medium as compared to the non-irradiated control (Fig. 3 A, B).At an irradiation of 100 Gy and 250 Gy, there were no differences on the solid medium, obtaining a clove-like culture for both tested strains.

By exposing the *Pseudomonas* C16 and *Pseudomonas aeruginosa* 3d strains to a 500 Gy irradiation, 300 UFC/ml for*Pseudomonas* C16 and 850 UFC/mlfor*Pseudomonas aeruginosa* 3d were obtained. At 1000 Gy irradiation, both strains showed 0 UFC/ml.

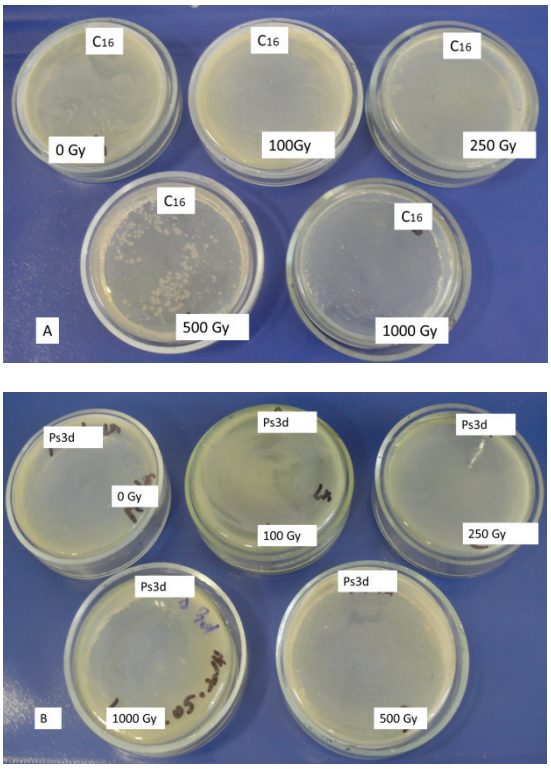

Figure 3. Development of *Pseudomonas* C16 (A) and *Pseudomonas aeruginosa* 3d (B) strains on solid LB medium after irradiation at different doses (100 Gy, 250 Gy, 500 Gy, 1000 Gy).

In terms ofthe influence of gamma radiation upon the ability of the *Pseudomonas* strains to synthesise bioproducts of interest (polyhydroxyalcanoates and biosurfactants), the results are promising.

The influence of gamma radiation on the synthesis of polyhydroxyalkanoates was assessed by cultivation of samples irradiated at different intensities in the Nile Red basal medium for 24 h, at a temperature of 28 °C and UV visualisation.

As it can be seen in Figure 4, the influence of gamma radiation (at the values tested by us) with respect to synthesis of polyhydroxyalkanoates,was not significant. At intensities of 50, 100, 250 Gy, both strains showed similar development to the control (non-irradiated strain), suggesting that gamma radiation had no quantitative stimulating effect, but mightboostquality.Samples irradiated with 1000 Gy and 2000 Gy, respectively, did not develop, thus confirming the lethal dose found by cultivating the irradiated samples in an LB environment.

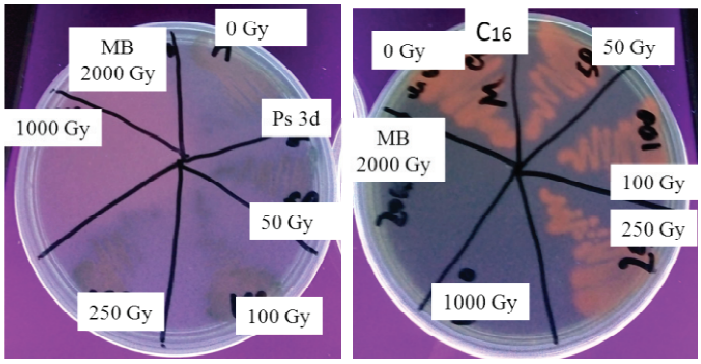

Figure 4. Influence of gamma radiation on the synthesis of polyhydroxyalkanoates.

Changes caused by gamma radiation on biosurfactant synthesis were assessed bythe oil spreading assay, a relevant test for surfactants (Fig. 5).Thus, an improvement of the oil spreading test forthe *Pseudomonas* C16 strain supernatant after irradiation with 100 Gy, 250 Gy and 200 Gy, respectively, was recorded as compared to the non-irradiated control (0 Gy).

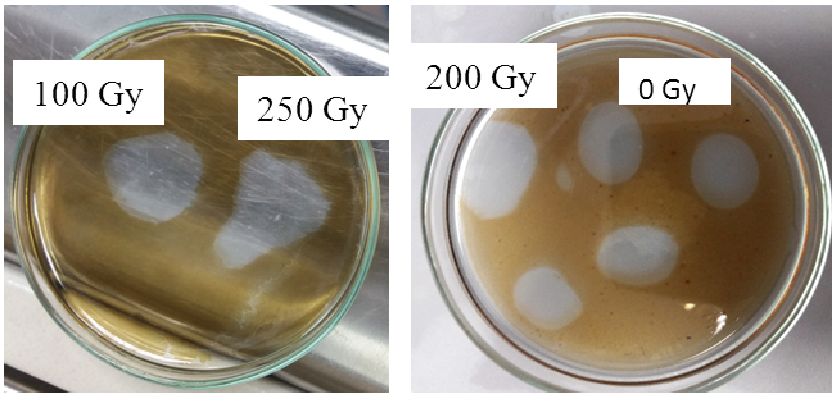

Figure 5. Influence of gamma radiation on the synthesis of surfactants.

The results obtained in the present study can provide a useful frame of reference, stimulating us to continue our research with this research approach.

CONCLUSIONS

Depending on the intensity of the radiation, changes in the growth rate were noticed. The higher the irradiation dose, the lower the development of samples. Both *Pseudomonas*strains are sensitive to the 1000Gy dose. This dose can be considered lethal.

The polyhydroxyalkanoate products under study did not show significant changes. The effect of gamma radiation at doses of 100 Gy, 250 Gy and 200 Gy, respectively, on *Pseudomonas* C16 strain surfactants was found to improve their quality, as determined by the oil spreading test.

ACKNOWLEDGMENT

This work was funded by the PN-III-P1-1.2-PCCDI2017-0323 Project “Utilisation of Gamma irradiation in biotechnological processes with applications in bioeconomy”.

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Received: April 10, 2019
Accepted: September 02, 2019