Etlik Vet Mikrobiyol Derg, 2024; **35 (1):** 90-95
doi: https://doi.org/10.35864/evmd.1380917

Review Article
Derleme

Effects of bacterial biodosimeters against radiation: A review study

Muhammet Arslan[1], Meltem Delimanlar[2*], Ahmet Koluman[3]

[1] *Pamukkale Üniversitesi, Tıp Fakültesi, Denizli, Türkiye*
[2,3.] *Pamukkale Üniversitesi, Teknoloji Fakültesi, Biyomedikal Mühendisliği Bölümü, Denizli, Türkiye*

Geliş Tarihi / Received: 30.10.2023, **Kabul Tarihi** / Accepted: 12.03.2024

Abstract: The bacterial biodosimeter is a type of biodosimeter that utilizes bacteria as the challenging organism to measure the effective dose of a reactor. These biodosimeters capitalize on the unique responses of bacterial systems to ionizing radiation, providing valuable insights into the biological effects of radiation, and enabling accurate dose estimation, and the potential health risks for living organisms. This review covers the details of the advantages and disadvantages of using bacteria for area monitoring of radiation and the current state of knowledge regarding bacterial biodosimeters. Additionally, methods for the detection of bacteria, protocols for radiation exposure, and factors that could influence culture conditions have been examined. This review aims to consolidate the existing knowledge on bacterial biodosimeters and stimulate further research to harness their full potential in radiation monitoring and protection.

Keywords: Biodosimeter; Bacterial biodosimeter; Exposure; Radiation; Optimization

Bakteriyel biyodozimetrelerin radyasyona karşı etkileri: Bir derleme çalışması

Özet: Bakteriyel biyodozimetre, reaktörün etkin dozunu ölçmek için bakterileri meydan okuma organizması olarak kullanan bir tür biyodozimetredir. Bu biyodozimetreler, bakteri sistemlerinin iyonlaştırıcı radyasyona karşı benzersiz tepkilerinden faydalanarak, radyasyonun biyolojik etkileri hakkında değerli bilgiler sunmakta ve doğru doz tahminini sağlamaktadır, aynı zamanda canlı organizmalar için potansiyel sağlık risklerini belirlemektedir. Bu derleme, radyasyonun alan izlemesi için bakterilerin kullanılmasının avantajları ve dezavantajlarının detaylarını ve bakteriyel biyodozimetreler hakkındaki mevcut bilgi durumunu kapsamaktadır. Ayrıca, bakterilerin tespit yöntemleri, radyasyon maruziyeti protokolleri ve kültür koşullarını etkileyebilecek faktörler incelenmiştir. Bu derleme, bakteriyel biyodozimetrelerin var olan bilgisini bir araya getirme ve radyasyon izleme ve koruma konularında potansiyellerini tam olarak kullanmak için daha fazla araştırmayı teşvik etmeyi amaçlamaktadır.

Anahtar kelimeler: Bakteriyel Biyodozimetre; Biyodozimetre; Maruziyet; Optimizasyon; Radyasyon

Introduction

According to the International Atomic Energy Agency (IAEA), some 23 million workers worldwide are are subjected to ionizing radiation as part of their job responsibilities (*Workers*, n.d.). In order to protect them against such an exposure, some specific steps as; regular monitoring, protective equipment, or countermeasures such as shielding can be followed (Boice et al., 2020). Additionally; training, information exchange, and consistent health surveillance are also important factors for an efficient occupational radiation protection regime (Albander, 2021). In the modern era of radiation exposure assessment and radiation protection, biodosimetry has emerged as a critical tool for accurately evaluating radiation doses and understanding their biological effects. Among the diverse array of biodosimetric approaches, bacterial biodosimeters have garnered increasing attention for their unique ability to provide reliable and versatile assessments of ionizing radiation exposure. These biodosensors capitalize on the distinct and specific responses of bacterial systems to radiation-induced damage, offering valuable insights into the radiation-induced biological effects and facilitating precise dose estimations(Heron et al., 2010).

The IAEA Occupational Radiation Protection Programme aims to promote and assist in establishing an internationally harmonized approach for optimizing occupational radiation protection(*International Conference on Occupational Radiation Protection – Strengthening Radiation Protection of Workers – Twenty Years of Progress and the Way Forward*, n.d.) . It offers direction to member nations of the International Atomic Energy Agency (IAEA) by means of a structured set of

Yazışma adresi / Correspondence: *Meltem Delimanlar, Yunusemre Mah. 6459 sokak No:10-13, Pamukkale, Denizli*
email: delimanlarmeltem@gmail.com

ORCID IDs of the authors: *[1]0000-0001-5565-0770 • [2]0000-0003-4152-7805 • [3]0000-0001-5308-8884*

safety standards, categorized into three levels: Safety Fundamentals, Safety Requirements, and Safety Guides(*International Conference on Occupational Radiation Protection – Strengthening Radiation Protection of Workers – Twenty Years of Progress and the Way Forward*, n.d.). In addition to that it also helps Member States to apply these standards and guidelines in practice through various activities, such as training, information exchange, technical cooperation, and peer review services. In the year of 2014, the IAEA developed the Occupational Radiation Protection Call-for-Action with several other international organizations, which includes nine key areas that require global attention in the field of radiation protection of workers (*Workers*, n.d.). These actions have significance on the safe use of ionizing radiation. The IAEA also releases e-learning courses on radiation protection of workers to enhance national capabilities and awareness (Suárez et al., 2001).

In 2014, the (IAEA), in collaboration with various international organizations, created the Occupational Radiation Protection Call-for-Action. This document was designed to tackle the deficiencies, obstacles, and advancements within the domain of protecting workers from radiation exposure(*Workers*, n.d.). It states nine key areas that require global attention, which are:

1. Implementing existing safety standards and guidance
2. Strengthening assistance to countries with less developed programs for occupational radiation protection
3. Improving safety culture among exposed workers and their management
4. Enhancing the protection of workers in medicine
5. Enhancing the protection of workers exposed to natural sources.
6. Enhancing the protection of workers in the nuclear fuel cycle and decommissioning activities
7. Enhancing the protection of workers in emergency exposure situations and existing exposure situations
8. Convening international forum for information exchange
9. Strengthening education, training, and qualification programs for occupational radiation protection professionals

In this regard, these actions plays a crucial role and are taken for the safe utilization of ionizing radiation and ensuring that workers receive sufficient protection from potential hazards.

Dosimeters and biodosimeters

A dosimeter is an instrument employed to measure an individual's exposure to ionizing radiation. Typically, dosimeters provide a report in the form of a dose, expressed as the absorbed radiation energy measured in grays (Gy) or the equivalent dose measured in sieverts (Sv). There are different types of dosimeters, such as film badge, thermoluminescent, electronic or ion-chamber dosimeters. Dosimeters possess the capability to offer a quantitative and reproducible measurement of absorbed dose by inducing alterations in one or more of their physical characteristics when subjected to ionizing radiation energy. Dosimeters are worn by people who work with or near sources of radiation, such as radiographers, nuclear power plant workers, doctors using radiotherapy, or HAZMAT workers (Yukihara et al., 2022).

Biodosimetry is a measurement of biological response as a surrogate for radiation dose. Biodosimetry serves the purpose of estimating the dose and, when possible, forecasting or reflecting the medically significant reaction, which refers to the biological outcomes resulting from that dose(De Deene, 2022). Biodosimetry can utilize changes induced in the individual by ionizing radiation, such as cytogenetic damage, gene expression, protein or metabolite levels, or physiological alterations (Macchione et al., 2022). Ideally, the changes should be specific for ionizing radiation, and the response should be unaffected by other factors (Sproull et al., 2017). Biodosimetry can be useful for medical management of radiological emergencies, such as mass casualty incidents or accidental exposures (Swartz et al., 2014).

Popular types of biodosimetry methods can be listed as;

1. Dicentric chromosome assay: This method measures the frequency of dicentric chromosomes, which are abnormal chromosomes with two centromeres, in peripheral blood lymphocytes. Dicentric chromosomes are specific for ionizing radiation and correlate well with radiation dose.
2. Cytokinesis-block micronucleus assay: This method measures the frequency of micronuclei, which are small fragments of chromosomes that are not incorporated into the daughter nuclei during cell division, in binucleated lymphocytes. Micronuclei can be induced by ionizing

radiation and other genotoxic agents (Wilkins et al., 2017).

3. γH2AX marker of DNA damage: This method measures the level of phosphorylated histone H2AX (γH2AX), which is a marker of DNA double-strand breaks, in lymphocytes or other cell types. γH2AX foci can be detected by immunofluorescence staining and microscopy or flow cytometry (Wilkins et al., 2017).
4. Electron paramagnetic resonance (EPR): This technique quantifies the levels of persistent free radicals in biological substances like tooth enamel, nail clippings, or bone, which are generated as a result of exposure to ionizing radiation. EPR can provide an estimate of cumulative radiation exposure over a long period of time (Swartz et al., 2014).

Biodosimetry methods can be standardized and validated by following the guidance and recommendations from relevant authorities, such as the IAEA or the Food and Drug Administration (FDA) (Sholom et al., 2022). These authorities provide criteria and procedures for the performance, quality assurance, calibration, and interpretation of biodosimetry methods. Biodosimetry methods can also be standardized and validated by comparing them with physical dosimetry methods, such as thermoluminescent dosimeters or mobile phone components (*Radiation Biodosimetry Medical Countermeasure Devices Guidance for Industry and Food and Drug Administration Staff*, 2016). Such comparisons can help verify and validate the dose reconstruction accuracy and reliability of biodosimetry methods. Biodosimetry methods can also be standardized and validated by participating in inter-laboratory comparisons or proficiency testing programmes, such as the WHO BioDoseNet, which can help evaluate and improve the technical competence and performance of biodosimetry laboratories (Sproull et al., 2017).

Bacterial biodosimetry

Bacterial biodosimetry which is a form of biodosimetry that employs bacteria as an organism of challenge to assess the radiation dose of a reactor by evaluating its capacity to render them nonfunctional (Sperle et al., 2023). In another term bacterial biodosimetry is a type of biodosimetry that uses bacteria as a challenge organism to measure the fluence of a reactor by determining its ability to inactivate them. Bacterial biodosimetry can be used to evaluate the performance of lab-scale flow-through ultraviolet water disinfection reactors, which are used to control biofouling. Bacterial biodosimetry can be standardized and validated by following the same principles and procedures as other biodosimetry methods, such as using appropriate challenge organisms, avoiding photo repair, reducing protractions, and minimizing cell absorption on labware (*Workers*, n.d.). Bacterial biodosimetry can also be standardized and validated by comparing it with physical dosimetry methods, such as thermoluminescent dosimeters or mobile phone components (Sproull et al., 2017). Bacterial biodosimetry can also be standardized and validated by participating in inter-laboratory comparisons or proficiency testing programs.

Widespreadly used bacteria as challenge organisms for biodosimetry are:

1. *Escherichia coli*: This is a common gram-negative bacterium that can be found in the human gut and in various environments. It is widely used as a model organism for molecular biology and biotechnology. It is also sensitive to UV-C irradiation and can be easily cultured and quantified (Nocker et al., 2018).
2. *Bacillus subtilis:* This is a gram-positive bacterium that can form endospores, and are highly resistant to environmental stresses, including UV radiation. It is often used as a reference organism for biodosimetry of UV disinfection of water. It can also be used to study the mechanisms of DNA repair and spore formation (Sperle et al., 2023).
3. *Aquabacterium citratiphilum*: This is a gram-negative bacterium that can form biofilms on various surfaces, such as pipes or membranes. It is relevant for biofouling control by UV disinfection of water. It can also be used to study the effects of UV irradiation on biofilm formation and detachment (Sperle et al., 2023).
4. *Deinococcus radiodurans:* This is a bacterium known for its remarkable ability to withstand ionizing radiation, ultraviolet radiation, oxidation, desiccation, and various other environmental pressures (Farci et al., 2022). *Deinococcus radiodurans* is a crucial model for studying the mechanisms of DNA damage and repair, redox regulation, and survival strategies in response to high-dose ionizing radiation (Farci et al., 2022). *Deinococcus radiodurans* can also be used for bioremediation of radioactive waste or heavy metal pollution, as it can degrade organic compounds and transform toxic metals (Liu et al., 2023). *Deinococcus radiodurans* can also be

genetically manipulated to enhance its biotechnological applications (Ghosal et al., 2005).

Bacterial biodosimetry for area monitoring

Bacteria can be used for area monitoring of radiation by measuring their survival or inactivation rate after exposure to different doses of radiation. The same situation is also valid if their DNA damage or repair is measured after the exposure. Bacteria can also be used for area monitoring of radiation by measuring their growth rate or metabolic activity under different environmental conditions (Zhang et al., 2018). Bacteria can provide a biological indicator of the radiation level and the potential health effects on living organisms. Bacteria can also be used for bioremediation of radioactive waste or heavy metal pollution by degrading organic compounds or transforming toxic metals (*Workplace Monitoring - Home*, n.d.).

There are significant advantages of using bacteria for area monitoring. Bacteria are easy to culture, handle, and transport. Bacteria have a fast growth rate and a short generation time, which allows for rapid and repeated measurements. Bacteria have a high sensitivity and specificity to radiation, which enables accurate and reliable dose estimation. Bacteria have a wide range of radiation resistance and metabolic diversity, which allows for the selection of suitable strains for different radiation sources and environmental conditions. Bacteria can provide information on the biological effects of radiation, such as DNA damage, repair, mutation, and cell death.

On the contrary; disadvantages of using bacteria for area monitoring of radiation also exists. Bacteria may have different responses to radiation depending on their physiological state, growth phase, culture medium, and environmental factors. Bacteria may have different responses to radiation depending on the type, energy, dose rate, and quality of radiation. Bacteria may have different responses to radiation depending on the presence of other stressors, such as temperature, pH, oxygen, nutrients, or chemicals. Bacteria may have different responses to radiation depending on the interaction with other microorganisms or host cells. Bacteria may have limitations in detecting low doses or chronic exposures of radiation.

The technic to be applied for bacterial biodosimetry

To highlight the technic of standard culture conditions, irradiation protocols, and detection methods for bacteria some parameters should be taken into account.

Culture conditions; bacteria can be cultured in liquid or solid media with appropriate nutrients, pH, temperature, and oxygen levels (Wang & Salazar, 2015). The culture conditions should be optimized for the growth and survival of the selected bacterial strain and consistent for each experiment (Wang & Salazar, 2015). The culture conditions can be affected by the physiological state, growth phase, culture medium, and environmental factors of the bacteria. For example, the bacterial growth rate, metabolism, and resistance to radiation may vary depending on the nutrient availability, pH, temperature, oxygen level, and presence of other microorganisms or chemicals in the culture medium (Wang & Salazar, 2015).

Irradiation protocols; bacteria can be irradiated with different sources and doses of radiation, such as gamma rays, X-rays, or ultraviolet rays. The irradiation protocols should be standardized for the type, energy, dose rate, and quality of radiation and calibrated with physical dosimeters. The irradiation protocols should also minimize the exposure time and the variation of radiation dose across the sample. The irradiation protocols can be affected by the type, energy, dose rate, and quality of radiation and the sample matrix. For example, the radiation dose and the biological effects may vary depending on the source and energy of radiation, the exposure time and distance, the shielding and attenuation of radiation, and the composition and geometry of the sample.

Detection methods; bacteria can be detected and quantified by different methods, such as nucleic acid-based methods (e.g., PCR, hybridization), immunological methods (e.g., ELISA, immunofluorescence), biosensor methods (e.g., electrochemical, optical), or phenotypic methods (e.g., colony counting, turbidity). The detection methods should be sensitive, specific, accurate, and reliable for the target bacteria and compatible with the sample matrix. The detection methods can be affected by the sensitivity, specificity, accuracy, and reliability of the method and the sample matrix. For example, the detection limit, signal-to-noise ratio, false positive or negative results, and reproducibility may vary depending on the target bacteria, the detection principle and technique, the calibration and quality control of the method, and the interference or inhibition of the sample (Wise, 2006).

Conclusion

The conclusion of this review is that bacterial biodosimetry is a useful and promising technique for area monitoring of radiation, but it also has some challenges and limitations that need to be addressed. Bacterial biodosimetry requires standardization and validation of the culture conditions, irradiation protocols, and detection methods for bacteria, as well as the consideration of the factors that can influence the bacterial responses to radiation. Bacterial biodosimetry can provide information on the biological effects of radiation and the potential health risks for living organisms. Bacterial biodosimetry can also be integrated with other methods for radiation detection and bioremediation. There are some possible future directions for bacterial biodosimetry including, developing novel and improved culture conditions, irradiation protocols, and detection methods for bacteria that are more sensitive, specific, accurate, and reliable. The knowledge of exploring the molecular and cellular mechanisms of bacterial responses to radiation, such as DNA damage and repair, redox regulation, and survival strategies not only enhances our comprehension of the biological effects of radiation but also holds potential for the development of advanced radiation protection strategies. As we advance towards a deeper comprehension of bacterial biododosimeters, these invaluable tools will continue to play a pivotal role in safeguarding human health and enhancing radiation protection measures in various fields, including healthcare, space exploration, and emergency preparedness. Establishing and expanding the collaboration and communication among researchers, regulators, and stakeholders in the field of bacterial biodosimetry will require continued research and interdisciplinary collaboration.

References

Albander, H. (2021). Occupational health and radiation Safety of radiography workers. In *IntechOpen eBooks*. https://doi.org/10.5772/intechopen.95061

Boice, J. D., Dauer, L. T., Kase, K. R., Mettler, F. A., & Vetter, R. J. (2020). Evolution of radiation protection for medical workers. *British Journal of Radiology*, *93*(1112), 20200282. https://doi.org/10.1259/bjr.20200282

De Deene, Y. (2022). Radiation Dosimetry by Use of Radiosensitive Hydrogels and Polymers: Mechanisms, State-of-the-Art and Perspective from 3D to 4D. *Gels*, *8*(9), 599. https://doi.org/10.3390/gels8090599

Farci, D., Haniewicz, P., & Piano, D. (2022). The structured organization of *Deinococcus radiodurans'* cell envelope. *Proceedings of the National Academy of Sciences of the United States of America*, *119*(45). https://doi.org/10.1073/pnas.2209111119

Ghosal, D., Omelchenko, M. V., Gaidamakova, E. K., Matrosova, V. Y., Василенко, A. T., Venkateswaran, A., Zhai, M., Kostandarithes, H. M., Brim, H., Makarova, K. S., Wackett, L. P., Fredrickson, J. K., & Daly, M. J. (2005). How radiation kills cells: Survival of*Deinococcus radiodurans*and*Shewanella oneidensis*under oxidative stress. *Fems Microbiology Reviews*, *29*(2), 361–375. https://doi.org/10.1016/j.fmrre.2004.12.007

Heron, J. L., Padovani, R., Smith, I., & Czarwinski, R. (2010). Radiation protection of medical staff. *European Journal of Radiology*, *76*(1), 20–23. https://doi.org/10.1016/j.ejrad.2010.06.034

International Conference on Occupational Radiation Protection – Strengthening Radiation Protection of Workers – Twenty years of progress and the Way forward. (n.d.). https://www.iaea.org/events/occupational-radiation-protection-2022

Liu, F., Li, N., & Zhang, Y. (2023). The radioresistant and survival mechanisms of Deinococcus radiodurans. *Radiation Medicine and Protection*, *4*(2), 70–79. https://doi.org/10.1016/j.radmp.2023.03.001

Macchione, M. A., Páez, S. L., Strumia, M. C., Valente, M., & Mattea, F. (2022). Chemical Overview of gel Dosimetry Systems: A Comprehensive Review. *Gels*, *8*(10), 663. https://doi.org/10.3390/gels8100663

Nocker, A., Shah, M. S., Dannenmann, B., Schulze-Osthoff, K., Wingender, J., & Probst, A. J. (2018). Assessment of UV-C-induced water disinfection by differential PCR-based quantification of bacterial DNA damage. *Journal of Microbiological Methods*, *149*, 89–95. https://doi.org/10.1016/j.mimet.2018.03.007

Radiation Biodosimetry Medical Countermeasure Devices Guidance for Industry and Food and Drug Administration Staff. (2016, April). U.S. Food And Drug Administration. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/radiation-biodosimetry-medical-countermeasure-devices

Sholom, S., McKeever, S., Escalona, M., Ryan, T. L., & Balajee, A. S. (2022). A comparative validation of biodosimetry and physical dosimetry techniques for possible triage applications in emergency dosimetry. *Journal of Radiological Protection*, *42*(2), 021515. https://doi.org/10.1088/1361-6498/ac5815

Sperle, P., Khan, M. S., Drewes, J. E., & Wurzbacher, C. (2023). A practical bacterial biodosimetry procedure to assess performance of Lab-Scale flow-through ultraviolet water disinfection reactors. *ACS ES&T Water*, *3*(8), 2130–2139. https://doi.org/10.1021/acsestwater.2c00648

Sproull, M., Camphausen, K., & Koblentz, G. D. (2017). Biodosimetry: a future tool for medical management of radiological emergencies. *Health Security*, *15*(6), 599–610. https://doi.org/10.1089/hs.2017.0050

Suárez, R. C., Gustafsson, M., & Mrabit, K. (2001). IAEA Occupational Radiation Protection Programme. *Radiation Protection Dosimetry*, *96*(1), 17–20. https://doi.org/10.1093/oxfordjournals.rpd.a006575

Swartz, H. M., Flood, A. B., & Williams, B. B. (2014). Overview of the principles and practice of biodosimetry. *Radiation and Environmental Biophysics*, *53*(2), 221–232. https://doi.org/10.1007/s00411-014-0522-0

Wang, Y., & Salazar, J. K. (2015). Culture-Independent rapid detection methods for bacterial pathogens and toxins in food matrices. *Comprehensive Reviews in Food Science and Food Safety*, *15*(1), 183–205. https://doi.org/10.1111/1541-4337.12175

Wilkins, R., Rodrigues, M. A., & Beaton-Green, L. A. (2017). The application of Imaging Flow Cytometry to High-Throughput Biodosimetry. *Genome Integrity*, *8*. https://doi.org/10.4103/2041-9414.198912

Wise, K. (2006). Preparing Spread Plates Protocols. *Am. Soc. Microbiol. Microbe Libr.* https://www.asmscience.org/content/education/protocol/protocol.3085

Workers. (n.d.). https://www.iaea.org/topics/radiation-protection/workers. [Accessed:Sep. 30, 2023].

Workplace Monitoring - home. (n.d.). https://nucleus.iaea.org/sites/orpnet/training/workplacemonitoring/SitePages/Home.aspx

Yukihara, E., McKeever, S., Andersen, C., Bos, A., Bailiff, I., Yoshimura, E. M., Sawakuchi, G. O., Bossin, L., & Christensen, J. B. (2022). Luminescence dosimetry. *Nature Reviews Methods Primers*, *2*(1). https://doi.org/10.1038/s43586-022-00102-0

Zhang, X., Jiang, X., Yang, Q., Wang, X., Zhang, Y., Zhao, J., Qu, K., & Zhao, C. (2018). Online Monitoring of Bacterial Growth with an Electrical Sensor. *Analytical Chemistry*, *90*(10), 6006–6011. https://doi.org/10.1021/acs.analchem.8b01214