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Exploration of the interaction between antimicrobial peptides and gamma radiation for biosensor development.

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Abstract: The continuous advancement of nuclear science, associated with biotechnology, has provided innovative interdisciplinary approaches to overcome challenges related to the detection of ionizing radiation. This study investigates the use of antimicrobial peptides (AMPs), present in the total extract of the bacterium *Paenibacillus polymyxa* RNC-D, as a potential sensitive element in the development of gamma radiation biosensors. The total extract (TE) containing the AMPs was irradiated in a Gamacell 220 irradiator with a Co-60 source at the Radiation Technology Center (CTR) of IPEN, subjected to doses of 0.5, 1.0, 4.0, and 10.0 Gy under controlled conditions. The evaluation of antimicrobial activity included tests against clinically relevant microorganisms, such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Listeria monocytogenes* and *Candida albicans*. The results revealed that the AMPs, particularly the peptide from *Paenibacillus polymyxa* RNC-D, denominated PpRNC-D, present in the fermentative extract, maintained antimicrobial activity after exposure to doses of 0.5 Gy and 1.0 Gy, especially against Gram-positive bacteria. However, a considerable loss of activity was observed at doses of 4.0 Gy and 10.0 Gy. These findings indicate that, although AMPs demonstrate changes in activity as a function of dose and could be explored for biosensing, their functional stability is limited at higher radiation doses. This work highlights the specific potential of these AMPs as a basis for portable and economical biosensors, applicable in environmental monitoring and radiological safety within certain dose ranges.

Keywords: Gamma Radiation, Biosensor, Peptide, Radiobiology.

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Exploração da interação entre peptídeos antimicrobianos e radiação gama para o desenvolvimento de biossensores.

Resumo: O avanço contínuo da ciência nuclear associado à biotecnologia tem proporcionado abordagens interdisciplinares inovadoras para superar desafios relacionados à detecção de radiação ionizante. Este estudo investiga o uso de peptídeos antimicrobianos (AMPs), presentes no extrato total da bactéria *Paenibacillus polymyxa* RNC-D, como potencial elemento sensível no desenvolvimento de biossensores de radiação gama. O extrato total (TE) contendo os AMPs foi irradiado em um irradiador Gamacell 220 com fonte de Co-60 no Centro de Tecnologias das Radiações (CTR) do IPEN, submetido as doses de 0,5, 1,0, 4,0 e 10,0 Gy sob condições controladas. A avaliação da atividade antimicrobiana incluiu testes contra microrganismos clinicamente relevantes, como *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Listeria monocytogenes* e *Candida albicans*. Os resultados revelaram que os AMPs, com destaque para o peptídeo da *Paenibacillus polymyxa* RNC-D, denominado PpRNC-D, presente no extrato fermentativo, mantiveram atividade antimicrobiana após exposição às doses de 0,5 Gy e 1,0 Gy, especialmente contra bactérias Gram-positivas. Contudo, observou-se uma perda considerável de atividade nas doses de 4,0 Gy e 10,0 Gy. Estes achados indicam que, embora os AMPs demonstrem alterações na atividade em função da dose, podendo ser explorados para biossensoriamento, sua estabilidade funcional é limitada em doses mais elevadas de radiação. Este trabalho ressalta o potencial específico destes AMPs como base para biossensores portáteis e econômicos, aplicáveis em monitoramento ambiental e segurança radiológica dentro de determinadas faixas de dose.

Keywords: Radiação Gama, Biossensor, Peptideo, Radiobiologia.

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1. INTRODUCTION

Radiobiology and biotechnology have registered notable advancements, enabling innovative solutions for challenges in ionizing radiation monitoring. Despite being widely used, traditional detection methods, such as Geiger-Müller detectors and thermoluminescent dosimeters, may present limitations, particularly concerning cost and operational complexity (Tauhata et al., 2014). In this context, biosensors emerge as potentially disruptive technologies, that offer greater precision, speed, and portability. These devices employ biological materials, such as enzymes or peptides, that respond selectively to specific stimuli, permitting real-time detection with high sensitivity (D'Orazio, 2011).

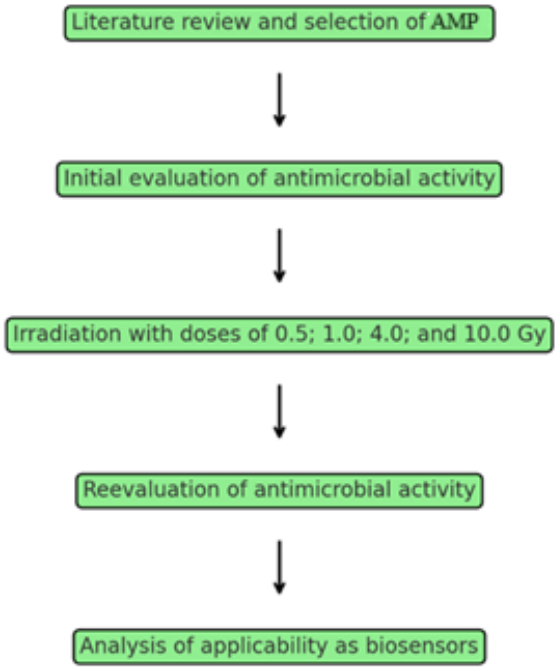
In national defense, the integration of biosensors into systems aimed at CBRN (Chemical, Biological, Radiological, and Nuclear) threats could represent a strategy to enhance security and achieve national sovereignty. Among the biological materials with potential for these applications, antimicrobial peptides, such as those derived from the endophytic bacterium *Paenibacillus polymyxa*, are of interest due to their antimicrobial properties and possible stability under certain conditions (Serrano, 2014; De Lima et al., 2022). A specific peptide of this origin, designated PpRNC-D, is investigated in this context. The hypothesis is that, by undergoing detectable alterations when exposed to radiation, these peptides could serve as components for innovative biosensors. Furthermore, their potential for incorporation into portable and economically viable devices could expand their utility across various fields, including radioprotection, national security, and environmental monitoring.

Therefore, in this study, the effects of gamma radiation on a crude extract containing peptides derived from the fermentation of *Paenibacillus polymyxa* were analyzed, with the objective of exploring its behavior in response to radiation and discussing its potential as an element in biosensors for applications in radioprotection, national security, and environmental monitoring.

2. MATERIALS AND METHODS

The methodological approach of this study was designed in sequential stages to investigate the effects of gamma radiation on the antimicrobial activity of a crude total extract (TE) from the bacterium *Paenibacillus polymyxa*, and it is detailed below in Figure 1.

Figure 1: Overview of the experimental process.



Source: Prepared by the author, 2024.

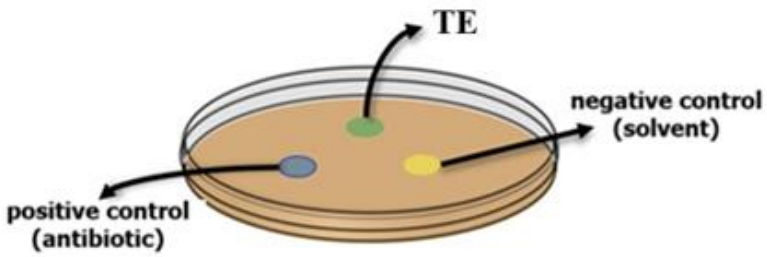
The selection of antimicrobial peptides (AMPs) for this study was preceded by a detailed literature review, involving the analysis of scientific articles and books on platforms such as PubMed, Scopus, and Web of Science. The search encompassed publications between 2010 and the present, prioritizing AMPs with potential for stability against ionizing radiation. Those produced by aerobic or facultative anaerobic bacteria capable of forming stress-resistant endospores were highlighted. These microorganisms were identified in diverse environments, including soil, water, and plant rhizospheres, demonstrating ecological and biotechnological relevance. Furthermore, selection criteria considered the viability of large-scale synthesis and cost-effectiveness, aiming to meet industrial and technological

demands in biosensor applications. It was decided to work with a crude extract from the bacterium *Paenibacillus polymyxa* RNC-D, known to produce the PpRNC-D peptide, as described by Serrano (2014).

Following the selection of the antimicrobial peptide, an evaluation of the antimicrobial activity of the crude total extract (TE) containing AMPs was performed. The TE was obtained from the supernatant of the bacterial culture (in YPM medium, 30°C, 18h), after centrifugation and filtration (0.22 μm membrane), resulting in a concentration of 1,26×104 μg/mL (lyophilized) and stored at -4°C.

The antimicrobial activity of the TE (10 μL per 6 mm well) was tested by the agar diffusion method, as illustrated in Figure 2, following guidelines from the Clinical and Laboratory Standards Institute (CLSI), against Gram-positive strains (*Staphylococcus aureus* ATCC 25922 and *Listeria monocytogenes* ATCC 15313), Gram-negative strains (*Escherichia coli* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 14207), and the fungus (*Candida albicans* ATCC 10231). Inocula were standardized (0.5 McFarland) and diluted (10^6 CFU/mL for bacteria and 10^5 CFU/mL for *C. albicans*). A commercial antibiotic solution was used as a positive control and PBS as a negative control, with incubation and halo measurements according to standard protocols, in triplicate.

Figure 2: Method used in the agar diffusion test, representing the TE well (green), the negative control (yellow), and the positive control (blue).



Source: Prepared by the author, 2024.

Following irradiation, the antimicrobial activity of the TE was reassessed to verify possible alterations. The comparative analysis of pre- and post-irradiation results allowed for the evaluation of gamma radiation's influence on the antimicrobial properties of the crude total extract.

The crude total extract containing the antimicrobial peptides was irradiated with different doses of gamma radiation (0.5, 1.0, 4.0, and 10.0 Gy) using a Cobalt-60 source at the Radiation Center Laboratory of IPEN/CNEN. Irradiation conditions were controlled to ensure experimental reproducibility, with the source activity in March 2024 at 368.945 Ci, a dose rate of 317.88 Gy/h, and a temperature of 26°C. To ensure experimental reproducibility, the irradiation chamber was maintained under strictly controlled temperature conditions (≤23°C) and a fixed, previously calibrated dose rate. The peptide samples, conditioned in 1.5 mL microtubes, were uniformly arranged in the irradiation chamber to ensure homogeneous exposure to radiation, as presented in Figure 2.

After each irradiation, the samples were immediately stored at -4°C and transported in a thermal box with reusable ice packs for the antimicrobial activity assays

Figure 3: Samples in the Co-60 irradiator - GammaCell 220.

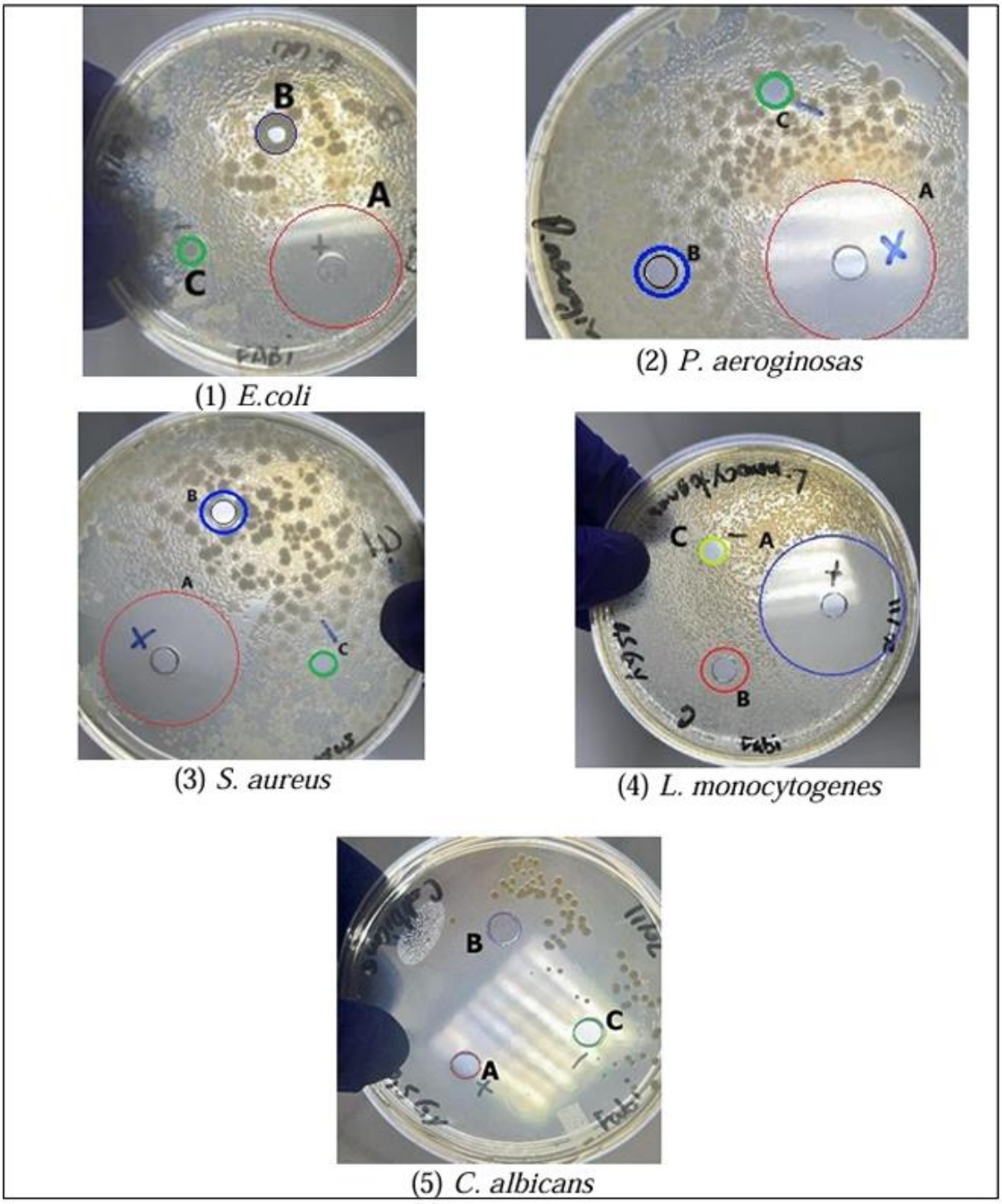
Source: Prepared by the author, 2024.

3. RESULTS AND DISCUSSIONS

The experiments conducted suggest that the TE from *Paenibacillus polymyxa*, containing AMPs such as PpRNC-D, exhibits a response to gamma radiation that is dose-dependent, which may be relevant for future investigations into gamma radiation biosensors. The literature indicates that the PpRNC-D peptide, isolated from *P. polymyxa* RNC-D, possesses antimicrobial activity and that its simplified structure could confer some stability in ionizing environments (Serrano, 2014; De Lima et al., 2022).

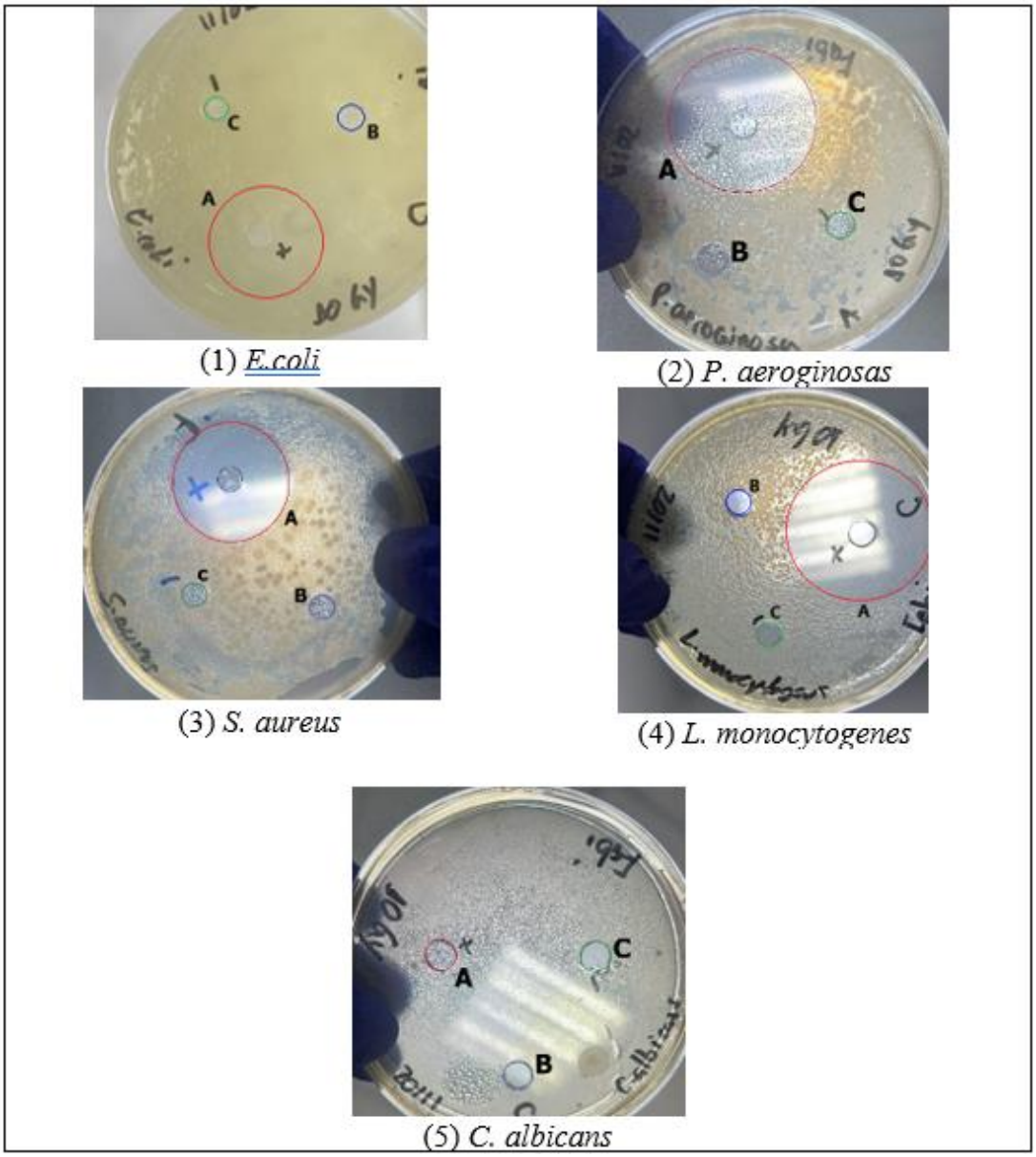
Evaluation of the extract's (TE) antimicrobial activity following gamma irradiation revealed a dose-dependent response. The TE maintained antimicrobial activity against *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, and *Pseudomonas aeruginosa* when irradiated at doses of 0.5 Gy and 1.0 Gy, as evidenced by consistent inhibition halos for these microorganisms (Figures 4 and 5). Specifically, at 0.5 Gy, the Gram-positive bacteria (*S. aureus* and *L. monocytogenes*) exhibited more pronounced inhibition halos compared to the Gram-negative *P. aeruginosa*, which, while also inhibited, displayed lower sensitivity. This observed difference in sensitivity may be attributed to structural variations in the cell walls of these bacterial types, affecting their interaction with antimicrobial agents.

Figure 4: Antimicrobial activity of TE irradiated at 0.5 Gy against (1) E. coli, (2) P. aeruginosa, (3) S. aureus, (4) L. monocytogenes, and (5) C. albicans. Controls: (A) antibiotic, (B) TE irradiated, (C) solvent.



Source: Prepared by the author, 2024.

Figure 5: Antimicrobial activity of TE irradiated at 1.0 Gy against (1) E. coli, (2) P. aeruginosa, (3) S. aureus, (4) L. monocytogenes, and (5) C. albicans. Controls: (A) antibiotic, (B) TE irradiated, (C) solvent.

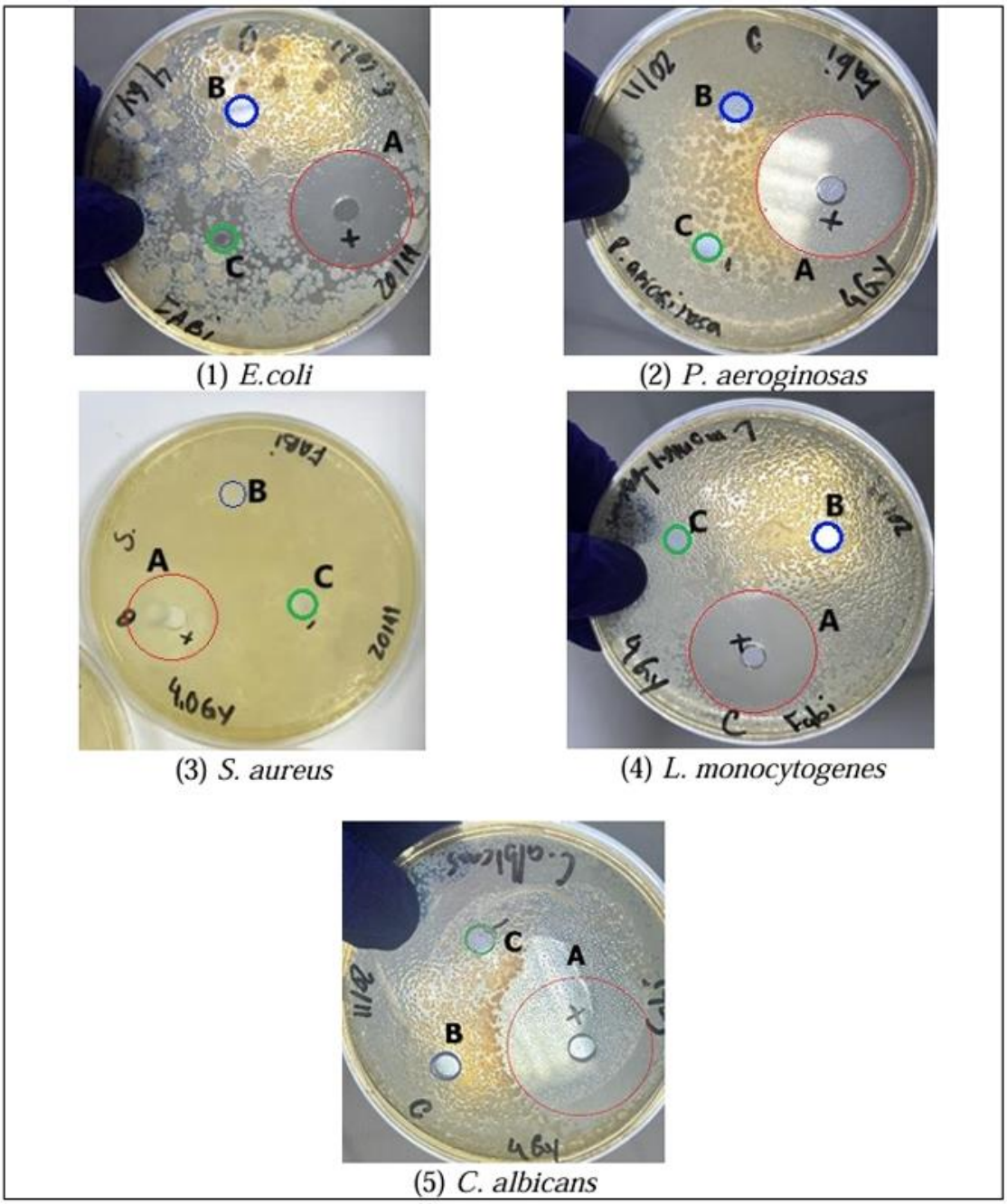


Source: Prepared by the author, 2024.

In contrast, at the higher doses of 4.0 Gy and 10.0 Gy, no inhibition halo was observed for any of the bacterial microorganisms tested, as demonstrated in Figure 6 and 7. This finding strongly suggests that exposure to these levels of gamma radiation significantly compromises the bioactive components of the TE responsible for antimicrobial activity, leading to their inactivation. The interaction of ionizing radiation with the aqueous medium

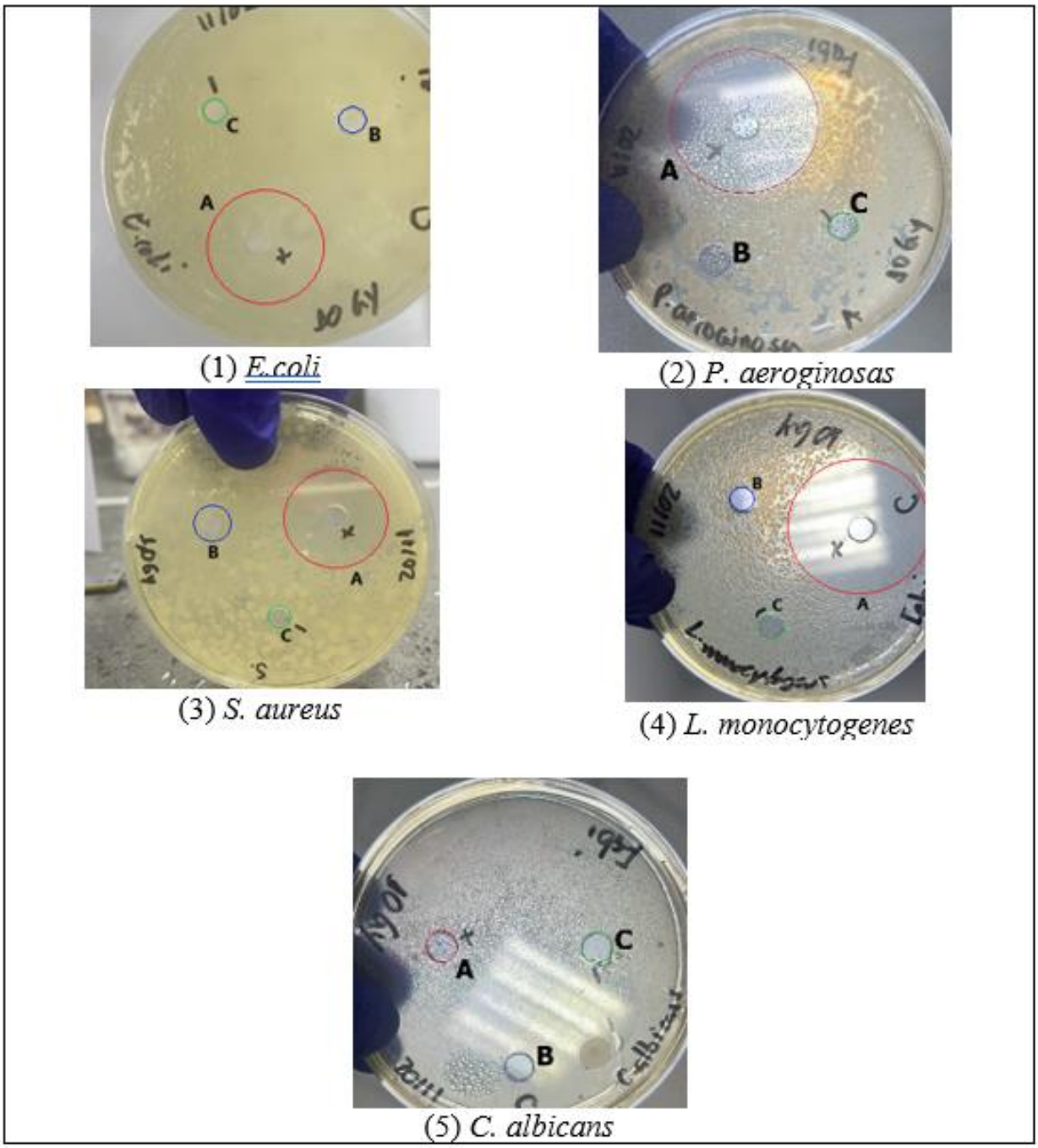
of the extract can lead to the formation of reactive oxygen species (ROS) in sufficient quantity to cause irreversible structural and functional changes in peptides and other metabolites present (Nardi, 2009; Silva et al., 2022).

Figure 6: Antimicrobial activity of TE irradiated at 4.0 Gy against (1) E. coli, (2) P. aeruginosa, (3) S. aureus, (4) L. monocytogenes, and (5) C. albicans. Controls: (A) antibiotic, (B) TE irradiated, (C) solvent.



Source: Prepared by the author, 2024.

Figure 7: Antimicrobial activity of TE irradiated at 10.0 Gy against (1) E. coli, (2) P. aeruginosa, (3) S. aureus, (4) L. monocytogenes, and (5) C. albicans. Controls: (A) antibiotic, (B) TE irradiated, (C) solvent.

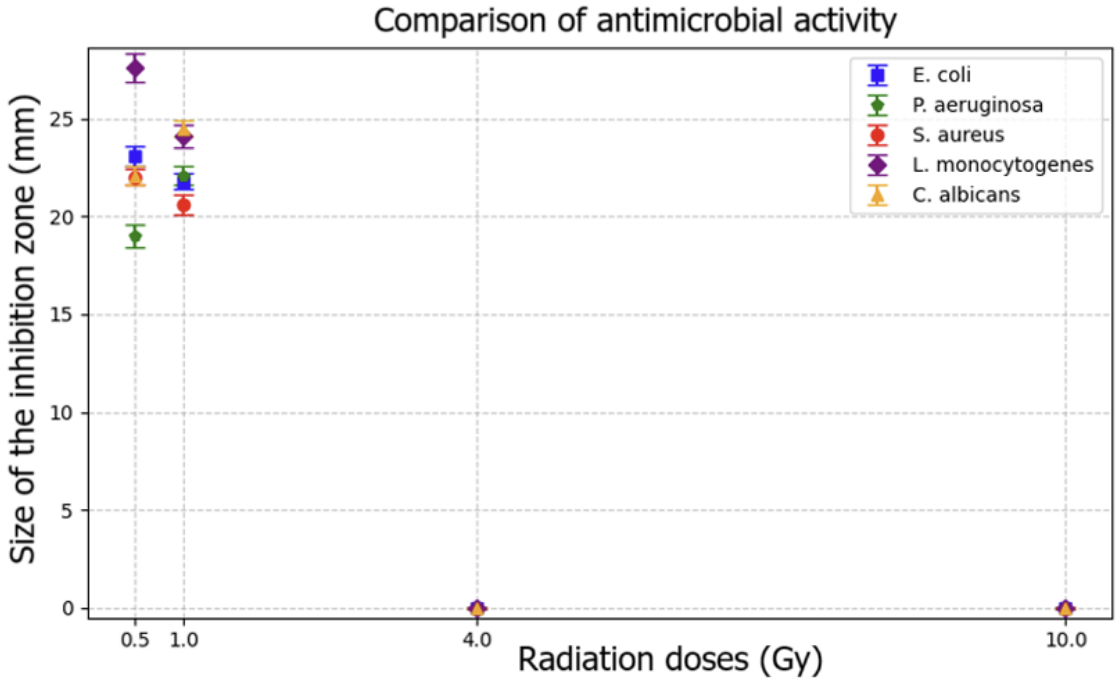


Source: Prepared by the author, 2024.

The results for *Candida albicans*, however, showed inconsistencies, not allowing for a clear correlation between radiation dose and antifungal activity. This inconsistency may have been influenced by factors such as the intrinsic heterogeneity of the fungal population or experimental variations during handling and incubation, which can be more critical for assays with yeasts compared to bacteria (De Cesare, 2020).

The maintenance of antimicrobial activity at doses of 0.5 Gy and 1.0 Gy, followed by its loss at higher doses, especially against bacteria as observed with *P. aeruginosa*, which showed lower sensitivity (but not an absence of inhibition at 0.5 and 1.0 Gy) to the irradiated TE, consistent with its known greater intrinsic resistance to various antimicrobial agents (Hing et al., 2022) suggests a stability threshold for the active components of the TE against gamma radiation. This information is better visualized in Figure 8, which demonstrates the relationship between the gamma radiation dose and the size of the inhibition halo for each tested pathogen.

Figure 8: Diameter of inhibition halos (mm) for E. coli (1), P. aeruginosa (2), S. aureus (3), L. monocytogenes (4), and C. albicans (5) as a function of gamma radiation dose (0.5 to 10.0 Gy).



Source: Prepared by the author, 2024.

This differential dose response could be explored in the conceptualization of biosensors, where the alteration in biological activity might be correlated with radiation exposure. However, inactivation at higher doses (≥ 4.0 Gy) represents an important limitation for applications in environments with more intense radiation levels.

Therefore, the applicability of these findings in the development of biosensors lies in the possibility of correlating the change in antimicrobial activity (or other physicochemical properties of the peptide that can be monitored) with the received radiation dose. Nevertheless, the loss of activity at higher doses is a limiting factor that needs to be considered.

The next step in this study would be experimental validation under conditions that more closely simulate real-world scenarios, evaluating the extract's applicability as an indicator of radiation exposure.

4. CONCLUSIONS

This study investigated the influence of gamma radiation on the antimicrobial activity of a crude total extract from *Paenibacillus polymyxa*, containing the PpRNC-D peptide. The results indicate that the antimicrobial activity of the extract is dose-dependent. Maintenance of inhibitory capacity against the tested bacterial microorganisms (*S. aureus*, *E. coli*, *L. monocytogenes*, *P. aeruginosa*) was observed after exposure to doses of 0.5 Gy and 1.0 Gy.

However, at higher doses, specifically 4.0 Gy and 10.0 Gy, the antimicrobial activity of the extract was notably absent, suggesting that the integrity or functionality of the antimicrobial components is compromised above a certain radiation dose threshold.

These findings demonstrate the stability of extracts containing AMPs in irradiated environments, suggesting their response to radiation merits further exploration. Specifically, the extract's ability to maintain antimicrobial functionality at doses up to 1 Gy, coupled with the alteration of this functionality as the dose increases, presents promising characteristics

for the development of biosensors. Future studies should therefore focus on identifying the exact mechanisms of AMP alteration by gamma radiation and on creating biosensor prototypes that leverage the relationship between radiation dose and peptide response. For broader applications, such as in environmental monitoring and public safety, the observed loss of activity at higher doses must also be carefully considered.

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CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

REFERENCES

[1] Alfaya, A. A. S., Kubota, L. T. A utilização de materiais obtidos pelo processo de Sol-Gel na construção de biossensores. Química Nova, v. 25, n. 5, pp. 835-841, 2002.

[2] Chilton, A. B., Shultis, J. K. and Faw, R. E. (1984). Principles of Radiation Shielding, Prentice-Hall.

[3] Choi, S. K. et al. Identification and functional analysis of the fusaricidin biosynthetic gene of Paenibacillus polymyxa E681. Biochem Biophys Res Commun, v. 365, pp. 89-95, 2007.

[4] CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 34th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, 2024.

[5] Conklin, James & Walker, Richard. (2009). Military Radiobiology. International Journal of Radiation Biology, INT J RADIAT BIOL. 52. 407

[6] De Cesare, G. B. et al. Antimicrobial Peptides: a New Frontier in Antifungal Therapy. mBio, v. 11, n. 6, pp. 02123-20, 2020.

[7] De Lima, M. R. et al. Estudo do limiar da eficiência funcional de peptídeo antimicrobiano (AMP) sujeito a irradiação gama. International Joint Conference Radio 2022, Poços de Caldas, pp. 363-364, 2022.

[8] D'Orazio, P. Biosensors in clinical chemistry. Clínica Chimica Acta, International Journal of Clinical Chemistry, pp. 1749-176, 2011.

[9] Gu, L. et al. Production of a newly isolated Paenibacillus polymyxa biocontrol agent using monosodium glutamate wastewater and potato wastewater. J Environ Sci, v. 22, n. 9, pp. 1407-1412, 2010.

[10] Huang, X. Y. et al. Exopolysaccharides of Paenibacillus polymyxa: a review. International Journal of Biological Macromolecules, v. 261, pp. 129663, 2024.

[11] Lal, S., Tabacchioni, S. Ecology and biotechnological potential of Paenibacillus polymyxa: a minireview. Indian Journal Microbiology, n. 49, pp. 2-10, 2009.

[12] Nardi, D. T. Estudo dos efeitos da radiação gama na estrutura de alguns peptídeos de relevância biológica. Universidade Federal de São Paulo, São Paulo, 2009.

[13] NCCLS. Método de Referência para Testes de Diluição em Caldo para a Determinação da Sensibilidade a Terapia Antifúngica das Leveduras; Norma Aprovada—Segunda Edição. Norma M27-A2 do NCCLS (ISBN 1-56238-469-4). NCCLS, Wayne, Pennsylvania, Estados Unidos, 2002.

[14] Okarvi, S. M., Maecke, H. R. Peptides for Nuclear Medicine Therapy: chemical properties and production. Therapeutic Nuclear Medicine, [S.L.], pp. 105-123, 2013.

[15] Serrano, N. F. G. Produção de compostos antimicrobianos por Paenibacillus polymyxa RNC-D: otimização das condições de cultivo, purificação e caracterização dos bioprodutos. Universidade Federal de São Carlos, 2014.

[16] Hing, J. N.; Jong, B. C.; Liew, P. W. Y.; Ellyna, R. E.; Shamsudin, S. Gamma Radiation Dose-Response of Gram-Positive and Gram-Negative Bacteria. Malaysian Applied Biology, v. 51, n. 5, p. 107–112, 2022.

[17] Tauhata, L., Salati, I., Di Prinzio, R., Di Prinzio, M. Radioproteção e Dosimetria: Fundamentos. 10ª ed. Rio de Janeiro: Instituto de Radioproteção e Dosimetria, CNEN, 2014.

[18] Thomazini, B. S. Desenvolvimento de biossensor óptico para detecção de microrganismos patogênicos. Universidade Federal de São Carlos, São Carlos, 2016.

[19] Wang, J. Electrochemical biosensors: towards point-of-care cancer diagnostics. Biosensors and Bioelectronics, v. 21, n. 10, pp. 1887-1892, 2006.

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