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EXPLORING THE INTERACTION BETWEEN ANTIMICROBIAL PEPTIDES AND GAMMA RADIATION FOR THE DEVELOPMENT OF BIOSENSORS

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ABSTRACT

Growing technological developments and challenges in the nuclear field require innovative approaches that integrate knowledge from different areas. The present study proposes a research uniting nuclear science and biotechnology in the investigation of the potential of antimicrobial peptides (PAMs) derived from the bacterium Paenibacillus polymyxa as materials sensitive to gamma ionizing radiation. The research was based on a comprehensive literature review and experimental evaluation of the activity of antimicrobial peptides (PAMs) derived from the bacterium Paenibacillus polymyxa before and after exposure to gamma radiation. The selected PAMs were exposed to different doses of gamma radiation (0.5, 1.0, 4.0 and 10.0 Gy) using a Gamacell 220 irradiator with a Co-60 source. The antimicrobial activity of the peptides was evaluated by the agar diffusion test, using a variety of microorganisms of clinical and environmental relevance, including Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Listeria monocytogenes and Candida albicans. These microorganisms were selected based on

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their medical importance as infection-causing agents, and because they represent different classes of pathogens (Gram-positive and Gram-negative bacteria). The results obtained through the literature review and preliminary tests allowed to evaluate the potential of PAMs as detector elements in biosensors and as antimicrobial agents in environments contaminated by radiation, due to their ability to generate detectable variations in their physicochemical properties when exposed to gamma ionizing radiation, allowing the rapid and accurate detection of gamma radiation levels. This innovative approach aims to overcome the limitations of traditional detection methods, which can be slow, expensive, and complex. The research was based on a review of the literature that identified promising peptides with significant antimicrobial activity in Paenibacillus polymyxa and the resistance of these PAMs to ionizing radiation, evaluating their efficiency threshold after exposure. The ability of PAMs to present a threshold to radiation and maintain their antimicrobial activity makes them ideal candidates for the development of portable and fast-response biosensors, with affordable cost, easy reproducibility, high sensitivity and other characteristics that are expected from an efficient biosensor, which can be applied in several areas from monitoring, such as environmental, civil protection, public security and national defense.

Keywords: Biotechnology. Gamma radiation. Antimicrobial Peptide. Biosensor.

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INTRODUCTION

Rapid technological advancement and challenges in the nuclear field require innovative solutions that integrate knowledge from diverse disciplines, especially in the context of nuclear science and biotechnology. Radiobiology, for example, plays a central role in understanding the effects of ionizing radiation on biological systems, which is crucial both for the enhancement of oncological therapies and for the protection of individuals exposed to potentially dangerous levels of radiation. On the other hand, biotechnology has allowed progress in the manipulation of organisms and molecules for various applications, including the development of biosensors, which has shown great potential in several areas, such as medicine, environment, nuclear safety and others (OKARVI and MAECKE, 2013).

In the field of ionizing radiation detection, in particular gamma radiation, accurate identification and effective monitoring are of utmost importance. This is especially relevant in scenarios involving nuclear safety, radioprotection, and environmental control. Traditional detection methods, such as thermoluminescent dosimeters and Geiger-Müller detectors, although widely used, have some inherent limitations, such as high costs, operational complexity, and low portability (ALFAYA and KUBOTA, 2002; ATTIX, 1986). In this context, biosensors emerge as promising alternatives, being devices that use biological components, such as proteins, enzymes, or cells, to detect and quantify specific substances with high precision.

An innovative approach in this field is the use of antimicrobial peptides (PAMs) as sensitive elements in biosensors. These peptides, composed of short chains of amino acids, are produced by a variety of organisms, including bacteria, fungi, and plants, and have antimicrobial activity against a wide range of microorganisms. Among the producing organisms, the bacterium *Paenibacillus polymyxa* has stood out as a promising source of PAMs with several biotechnological applications. Among the compounds produced by this bacterium, polymyxins, jolipeptin, polypeptins, gavaserin, saltavalin, gatavalin, fusaricidins, and polyxin stand out (CHOI et al., 2007), which act against a variety of bacteria (Gram-positive and Gram-negative) and fungi (GU et al., 2010). The diversity of PAMs produced by *P. polymyxa* demonstrates the biotechnological potential of this bacterium for the development of new antimicrobial compounds (SERRANO, 2014). However, the stability of these peptides under harsh conditions, such as exposure to ionizing radiation, is still poorly explored. A preliminary study conducted by De Lima et al. (2022) demonstrates the

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effect of gamma radiation on PAMs, paving the way for further investigations into the use of these peptides in applications involving radiation. Based on the literature review, the central hypothesis is that PAMs derived from *Paenibacillus polymyxa*, due to their structure and physicochemical properties, after exposure to gamma ionizing radiation, may present detectable changes in their properties, which emerge as a notable application in the development of biosensors for gamma radiation. In addition, the use of PAM-based biosensors can offer significant advantages over traditional methods, such as greater selectivity, lower production cost, and faster detection. This perspective is particularly relevant in emergency applications, such as the identification of radiation levels in nuclear accidents or real-time monitoring in risk areas (HUANG et al., 2024).

In summary, the use of antimicrobial peptides derived from *Paenibacillus polymyxa* as central elements in the development of biosensors for gamma radiation represents an innovative and promising approach. The integration of knowledge in nuclear science and biotechnology has the potential to generate significant advances in both radiation monitoring and nuclear safety, contributing to more effective solutions to global challenges. This proposal reflects the commitment to scientific and technological innovation, in line with the demands of a world in constant transformation.

METHODOLOGY

The methodology of this study was structured in stages that are planned to ensure the satisfactory result of the study.

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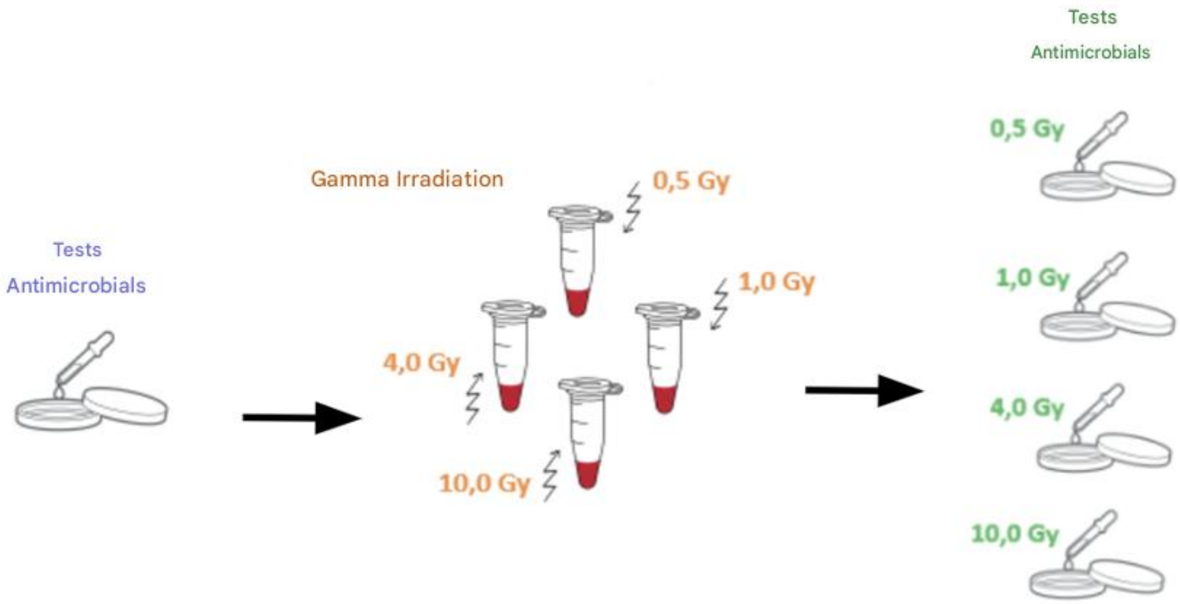
Figure 1. Simplified representation of the methodology.



Source: Author, 2024.

SELECTION AND OBTAINING OF PAMS

The selection of antimicrobial peptides (PAMs) for this study was preceded by a detailed literature review, involving the analysis of scientific articles, books and websites in the area of radiobiology, microbiology and biotechnology, published between 2010 and the present. The focus of the review prioritized the identification of PAMs with potential for stability against ionizing radiation, especially those produced by facultative aerobic or anaerobic bacteria that form stress-resistant endospores. These microorganisms are found in various environments, such as soil, water and in the rhizospheres of different plants (LAL and TABACCHIONI, 2009).

CHARACTERIZATION OF PAMS AND ANTIMICROBIAL ACTIVITY

After the selection of the antimicrobial peptide, the antimicrobial activity of the PAMs against different microorganisms was evaluated by means of microbiological assays using a variety of bacteria, including Gram-positive (*Staphylococcus aureus* ATCC 25922), Gram-negative (*Escherichia coli* ATCC 25923, *Pseudomonas aeruginosa* ATCC 14207, among others) and fungi (*Candida albicans* ATCC 10231). Distilled water was used as a negative control in order to verify that it does not interfere with the growth of the microorganism and as a positive control the antibiotic Penicillin (5000 units – 10mg/ml) to validate the technique and ensure that the microorganisms are sensitive. The antimicrobial activity of PAMs was evaluated using the agar diffusion technique, according to the

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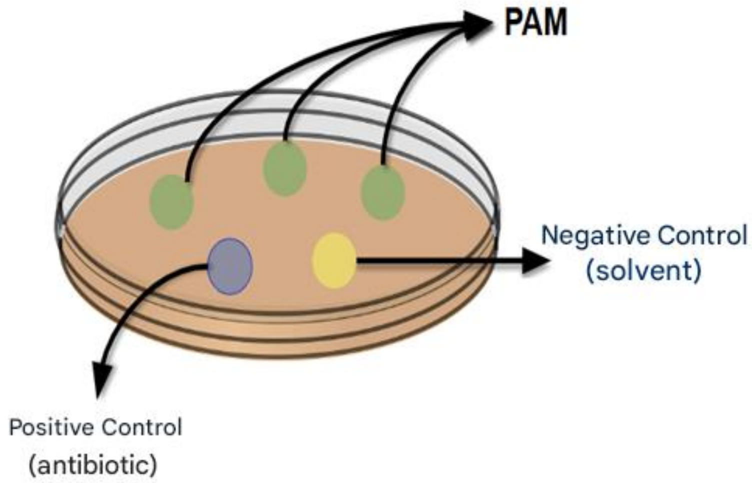
standards of the Clinical and Laboratory Standards Institute, 2024. After irradiation, the antimicrobial activity of the PAM was reassessed to verify possible alterations. The comparative analysis of the pre- and post-irradiation results will allow the evaluation of the sensitivity of PAMs to gamma radiation and identify possible changes in their properties.

Figure 2. Representation of the diffusion test on agar.



Source: Author, 2024.

IRRADIATION OF ANTIMICROBIAL PEPTIDES.

The antimicrobial peptides obtained were irradiated with different doses of gamma radiation, ranging from 0 to 10 Gy, using a Cobalt-60 source from the Radiation Center Laboratory of IPEN/CNEN shown in Figure 3. The irradiation conditions were controlled to ensure the reproducibility of the experiments, with source activity in March 2024 of 368.945 Ci, dose rate of 317.88 Gy/h and temperature of 26ºC. The samples were irradiated in a volume of 1.5 ml in Eppendorf tubes, performed in duplicates as shown in Figure 3.

Figure 3. Samples on the Co-60 irradiator - GamaCell 220.

Source: Author, 2024.

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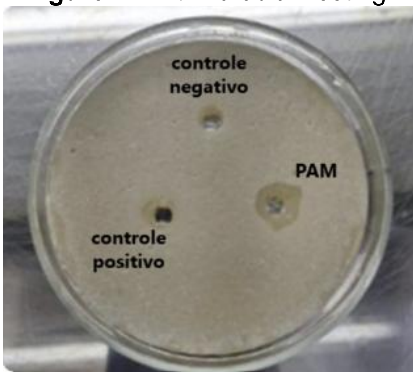
RESULTS

The literature review revealed that the antimicrobial peptide PpRNCD, derived from *Paenibacillus polymyxa*, emerged as a promising candidate for applications in gamma radiation biosensors, as highlighted by Serrano (2014) and De Lima et al. (2022). This choice is based on its unique structural and physicochemical properties, which provide high stability against ionizing radiation. A systematic search in databases such as PubMed and Scopus, in the last 10 years, was conducted to identify studies investigating the resistance of PAMs to ionizing radiation, which identified studies regarding peptides and gamma radiation, highlighting PpRNCD as a peptide with a high efficiency threshold after exposure to radiation.

Figure 4. Antimicrobial Testing.



Source: Author, 2024.

Table 1 presents the results of the antimicrobial activity of PpRNCD against several microorganisms. The data showed significant activity against Gram-positive bacteria (*Staphylococcus aureus*, *Listeria monocytogenes*) and fungi (*Candida albicans*), while no antimicrobial activity was observed against Gram-negative microorganisms such as *Pseudomonas aeruginosa*.

Table 1. Antimicrobial evaluation.

Microorganism	Antimicrobial activity
Pseudomonas aeruginosa	No
Listeria monocytogenes	No
Escherichia coli	Yes
Staphylococcus aureus	Yes
Candida albicans	Yes

Source: Author, 2024.

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DISCUSSION

The evaluation of the antimicrobial activity of antimicrobial peptides, after exposure to gamma radiation, corroborated the results obtained from studies that deal with the stability and maintenance of the biological activity of the antimicrobial peptide (ALFAYA and KUBOTA, 2002; SERRANO, 2014), indicating that the PpRNCD has a structural robustness that makes it highly stable even after exposure to gamma radiation. This stability is attributed to its simplified molecular structure, composed of five amino acids described by Serrano (2014), which minimizes oxidative damage caused by reactive oxygen species (ROS) generated by the interaction of radiation with water.

Ionizing radiation, when interacting with water, generates reactive oxygen species (ROS) which, in turn, interact with PAMs, causing oxidative damage and structural modifications (NARDI, 2009; HUANG et al., 2024). These changes result in modifications in the spectroscopic and electrochemical properties of the peptides, making them detectable by various analytical techniques corroborating the study by Thomazini (2016) that considers the concepts of photoluminescence in biomolecules, making it possible to investigate the application of different techniques currently used in the development of biosensors, including the immobilization of PAMs in appropriate platforms, such as electrodes or optical fibers.

Based on these results, it is possible to demonstrate the potential application of the antimicrobial peptide - object of this study - in the development of gamma radiation biosensors, in addition to the fact that it can use them in the microbiological remediation process, due to the antimicrobial effect (DE CESARE et al., 2020). Immobilized can be used as a biosensor, otherwise as a remediator controlling risks, demonstrating versatility. The focus on biosensors is due to their main characteristics of being accurate, portable, fast and low cost (WANG, 2006; D'ORAZIO, 2011), overcoming the limitations of traditional detection methods. The preliminary results of this study indicate that PAMs derived from *Paenibacillus polymyxa*, especially the peptide PpRNCD, are a possible and probable path for the development of gamma radiation biosensors.

CONCLUSION

This study concludes that the antimicrobial peptide PpRNCD, produced by *Paenibacillus polymyxa*, has dual and promising characteristics for application in the development of gamma radiation biosensors. The continuity of the experimental work should confirm its feasibility, enhancing the use of portable and fast biosensors for applications in nuclear safety and environmental monitoring. Therefore, the study aims to contribute to the advancement of knowledge in the areas of radiobiology, biotechnology, safety and nuclear instrumentation through innovation in the development of new detection methods through the investigation of the potential of antimicrobial peptides as materials sensitive to gamma radiation. Although resistance to microorganisms, which is the functionalization of PAMs, and the development of biosensors may seem like disconnected concepts at first. In the context of research, the ability of PAMs to maintain their antimicrobial activity even after radiation exposure is critical. This ensures that in environments with radiation, antimicrobial peptides continue to function as detector elements in the biosensor, without their function being compromised by the presence of microorganisms.

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