Paenibacillus polymyxa REPRESENTS A DUAL APPLICATIONS OF NATIONAL BIOPRODUCTS

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ABSTRACT

Paenibacillus polymyxa, a Gram-positive bacterium, exhibits dual applications in agricultural and pharmaceutical sectors due to its plant growth promotion, nitrogen fixation, and antimicrobial properties. Research focuses on its resistance to ionizing radiation (neutrons and gama), crucial for agricultural preservation and medical applications like radiotherapy. The antimicrobial peptide PAM PpRNCD, derived from P. polymyxa, shows promise in maintaining activity post-irradiation, offering potential in pharmaceutical and food industries. Utilizing nationally sourced bioinputs fosters sustainability and supports environmental protection. Encouraging research and production of products from national raw materials further strengthens market competitiveness and sustainability. *P. polymyxa* presents significant potential in industrial and environmental biotechnology, offering sustainable solutions for agriculture and pathogen control.

Keywords: *Paenibacillus polymyxa*. Antimicrobial peptides. Radiation resistance. Sustainability. Biotechnological applications.

1 INTRODUCTION

Paenibacillus polymyxa is a Gram-positive bacterium with sporulation capability, initially described by Prazmowski in 1880 and characterized by Ash et al. in 1994, as reported by the UK Food Standards Agency[1]. This bacterium has been the subject of numerous assays and research endeavors, ranging from taxonomic studies aimed at describing its properties and characteristics to biotechnological assays focused on activating and enhancing its specific functionalities.

Research published in scientific databases reveal that *P. polymyxa* offers various advantages and applications, especially in agriculture. Alves et al. (2020)[2] emphasize its plant growth-promoting properties and nitrogen fixation capacity. Due to these benefits, the bacterium is currently commercialized by several companies, including: Duwest Drokasa[3], LGC Standards[4], MarkNature[5], and Embrapa[6].

Apart from agricultural applications, *P. polymyxa* also exhibits significant antimicrobial properties. Serrano (2014)[7] in their thesis presented the antimicrobial activity of peptides extracted from *Paenibacillus polymyxa RNC-D*, a specific strain. In 2021, the researchers Soni, Nanjani and Keharia[8] published a paper on the genomic analysis and viability of the probiotic activity of the *Paenibacillus polymyxa HK4* strain. More recently, Ran et al. (2023)[9] reported the some extractions, with some funcionabilities like antibiotic and antifungal activity using the strain *Paenibacillus polymyxa 7F1*.

Considering the demands for nitrogen fixation (agricultural) and antimicrobial (pharmaceutical) properties expressed by this bacterium, research group at Instituto Militar de Engenharia - IME initiated assays aimed at verifying its resistance to directly ionizing (gamma) and indirectly ionizing (neutrons) radiation.

These studies, are through the Desenvolvimento e inovação de sensores, biossensores, detectores nacionais e produtos estratégicos relacionados a agentes QBRN de uso dual - PDI-QBRN Project, seek to ascertain the radiological resistance capacity of this bacterium and its products to assist in: (a) agriculture for planting and maintenance; (b) food exportation (where food irradiation methods may be applied for long-term preservation); (c) and in treatments and diagnostics utilizing radiation, such as cancer treatments. In this case, considering the frailty of patients and the frequent ineffectiveness of established pharmaceuticals, which are often inactivated due to the radiation present in treatment methods (HIGGINS et al., 2015)[10].

In the context presented, the exploration of *P. polymyxa* emerges as a promising biotechnological tool for enhancing agricultural sustainability and developing ecological solutions for pathogen control. This article aims to review and present new data on the studies and industrial and environmental applications of *P. polymyxa*, considering experiments aimed at verifying its resistance or non-resistance to radiation.

2 MATERIAL & METHODS

The materials utilized in this research include: (a) the antimicrobial peptide – PAM *PpRNCD*, extracted from the RNC-D strain by researcher Serrano[7] in 2014; (b) the *P. polymyxa* bacterium acquired from EMBRAPA[6] cuja coleta e isolamento do microrganismo foram realizados pela UFRJ – Universidade Federal do Rio de Janeiro (Federal University of Rio de Janeiro); (c)

the LAN – Laboratório de Análise por Ativação Neutrônica, and CETER – Centro de Tecnologia das Radiações in IPEN[11] - Instituto de Pesquisas Energéticas e Nucleares laboratories; (d) the Biotechnological Processes Laboratory at IME (LPB-IME); (e) and the benchtop bioreactor Bio-Tec-Twin from TECNAL[12]. Additionally, the following bacteria: *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Escherichia coli*, *Enterococcus faecalis* and *Staphylococcus aureus* and the fungus *Candida albicans*. The organisms will be used to verify the antibiotic effects of this peptide both before and after irradiation, as presented in Serrano[7] 2014 research and in our own research (radiation effects). Considering CNEN[13] – Comissão Nacional de de Energia Nuclear norm number 3.01, the occupational dose limits and permissible doses for the population are described in the Table 1.

Table 1: **CNEN. Norm nunber 3.01: The occupational dose limits and permissible doses for the population. Available at: https://www.gov.br/cnen/pt-br/acesso-rapido/normas/grupo-3/grupo3-nrm301.pdf. Accessed on: June 6, 2024)**

Limit of Annual Doses - CNEN			
Meansures	Organ	Occupationally Exposed Individual	General Public – Individual dose
Effective Dose	Full Body	20 mSv	1 mSv
Equivalent Dose	crystalline	20mSv	15 mSv
	Skin	500 mSv	50 mSv
	Hands and Feet	500 mSv	-

Therefore, for the experiments, a maximum dose of 50 mSv, a minimum dose of 0.5 mSv, and other intermediate doses were considered. The objective was to verify the resistance of biological materials according to the permitted variation of energy applied through radiation. The motivation for this research is the application of biological materials to support the inclusion and dissemination of nuclear applications in Brazil, whether for medical or pharmaceutical purposes, agriculture, or new uses.

The irradiation of the biological material was performed in the two laboratories of IPEN[11] and subsequently at IME, where the mentioned bacteria and fungi were plated in 80x15 mm Petri dishes. Specific culture media were used for the referred microorganisms.

The experiment consisted of verifying the antimicrobial activity before and after irradiation of the material - PAM PpRNCD. The test was conducted with one microorganism per day for the verification of antimicrobial activity. The selection was made following the priority order: highest occurrence, highest pathogenicity, Gram-negative organisms analyzed before Gram-positive ones, and finally, the fungus.

Following the triplicate methodology, one plate served as a negative control containing only the bacteria without the action of PAM *PpRNCD* from *Paenibacillus polymyxa*, and a blank control consisting of a petri dish with the culture medium for contamination control and analysis. In some subsequent research, other products and peptides produced by this bacterium can be analyzed by our group of researchers.

3 RESULTS & DISCUSSION

The analysis of the results obtained in the studies with the bacterium *Paenibacillus polymyxa* revealed notable characteristics as described previously by Alves et al. (2020)[2] and Serrano (2014)[8]. One significant research with the bioproducts from this referred bacteria was our analysis of the peptides' ability to maintain antimicrobial activity even after exposure to radiation. This result is particularly relevant in the context of both agricultural and pharmaceutical industries, supported by the growth of research and nuclear applications markets in Brazil (GOLDENBERG et al., 2010)[14].

The biomaterial's resistance to radiation suggests that its agricultural benefits can be preserved even in environments where food irradiation is used for preservation and pathogen control (Alves et al, 2020)[2]. Furthermore, in terms of health and well-being, it could potentially be applied as a drug to patients undergoing radiotherapy and radiodiagnostics (RIBEIRO and WANDERLEY, 2020)[15].

Additionally, the simplified molecular structure of the antimicrobial peptide *PAM PpRNCD*, derived from the *RNC-D* strain of *Paenibacillus polymyxa*, was highlighted as a significant factor in its radiation resistance. Composed of only five amino acids, this peptide may confer greater structural stability against ionizing radiation. This unique molecular characteristic may partially explain the observed ability of PAM PpRNCD to maintain its antimicrobial activity after irradiation, offering promising possibilities for pharmaceutical and food treatment applications.

The multifaceted application of these nationally sourced bioinputs underscores the importance of researching and appreciating national biological variability, which can be applied in various fields. Moreover, they serve as sustainable and biodegradable materials.

The protection of national fauna and flora, supported by R&D (Research and Development), is an essential component of environmental protection and resource management activities, as discussed in environmental accounting procedures and the CEPA and CREMA classifications (LINDMARK, 2019)[16]. It generates innovation and patents, which boost the national scene as well as the domestic market, enabling turnover and movement within the national market, as well as the utilization of raw materials from national sources, strengthening the three pillars of sustainability – social, economic, and environmental.

The bacterium Paenibacillus polymyxa and its products, whether nanobiomaterials or simply biomaterials, reinforce the need for research and encouragement of the production of products derived from national raw materials. In this way, not only do the research and methodologies open up national and international markets, but the products derived from them also do.

4 CONCLUSION

Paenibacillus polymyxa presents vast potential for applications in industrial and environmental biotechnology. Its ability to fix nitrogen, promote plant growth with biotechnological methods, and produce antimicrobial compounds make it a valuable tool for sustainable agriculture and biological control of pathogens. Its resistance to radiation should continue to be researched, enabling the exploration of other strains and by-products to produce materials that bridge the gap between nuclear - radioactive methods and biological solutions - in other words, more sustainable ones.

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