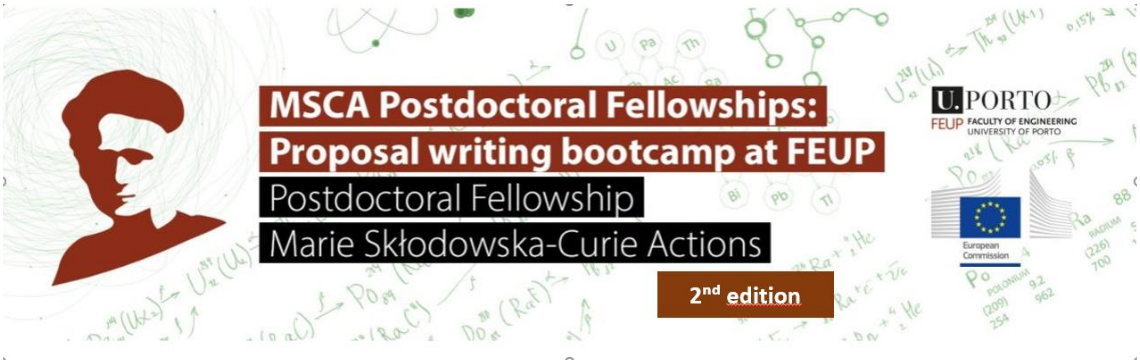

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PROJECT PROPOSAL	
Title (optional)	Multifunctional Therapeutic Biocomposites Combining *Macrocybe titans* Extracts and Borophosphate Glasses
Brief project description	

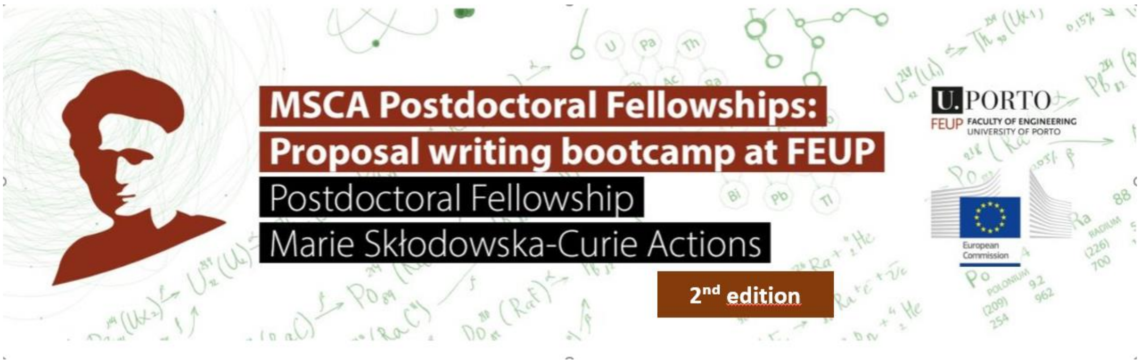
Introduction

The search for innovative and effective therapies to combat antimicrobial resistance (AMR) has become one of the most significant challenges in biomedical research. Fungi, in particular, exhibit remarkable metabolic plasticity (Choi & Kim, 2017; Nielsen et al., 2017), enabling the generation of diverse and underexplored chemical structures, including substances with antitumor, antioxidant, hepatoprotective, antibacterial, antidiabetic, and anti-inflammatory activities (Arrieche et al., 2023).

The species *Macrocybe titans* represents a singular example. Despite its striking morphology, the literature on *M. titans* is scarce, with only three publications. These include the identification of a fucogalactan polysaccharide with tumor cell migration-reducing activity (Milhorini et al., 2018), the discovery of macrocibin—a triglyceride capable of inhibiting tumor growth without affecting normal cells (Vilarino et al., 2020), and the characterization of fractions with antifungal activity against *Candida albicans* (Pereira et al., 2023). Building upon these findings, this study will focus on the antimicrobial potential of M. titans, aiming to explore its bioactive compounds as innovative solutions to combat antimicrobial resistance (AMR).

In parallel, the growing prevalence of antimicrobial resistance poses significant public health challenges. Immunocompromised patients, such as those in intensive care units or undergoing invasive procedures, are particularly susceptible to opportunistic infections caused by multidrug-resistant microorganisms such as *Escherichia coli* and methicillin-resistant *Staphylococcus aureus* (MRSA), both of which are classified as priority pathogens by the World Health Organization (WHO). This scenario, exacerbated by the overuse of antibiotics—especially during the COVID-19 pandemic—demands novel therapeutic approaches.

Integrating bioactive compounds from *M. titans* with advanced materials exhibiting antimicrobial properties, such as borophosphate glasses, emerges as a promising avenue. These glasses have been



shown to possess effective antimicrobial activity against various pathogens, including resistant strains like *S. aureus*, *E. coli*, and *Pseudomonas aeruginosa* (Saracini et al., 2021), making them promising candidates for the development of innovative therapeutic biocomposites.

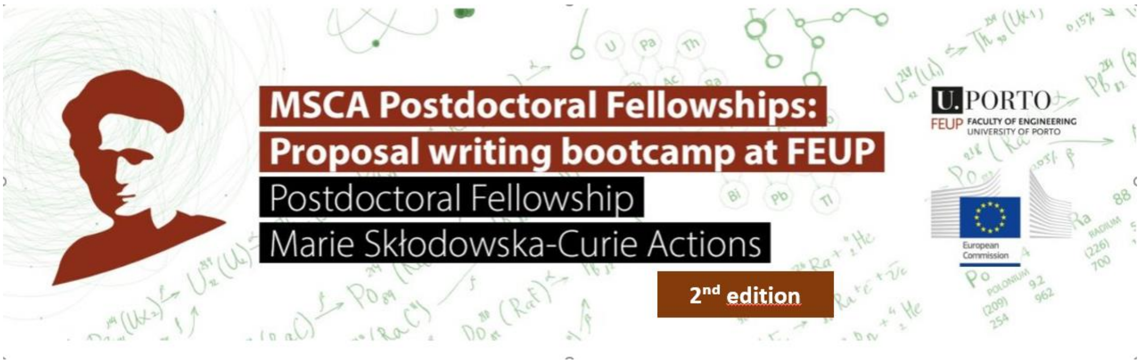
Building on these findings and addressing these pressing needs, the present project proposes the development of a therapeutic biocomposite integrating bioactive extracts of *M. titans* with borophosphate glasses. The objective is to create a multifunctional material capable of exerting antimicrobial effects and preventing bacterial adhesion on prosthetic surfaces. This approach aims to contribute to more effective and safer strategies for combating antimicrobial resistance, with potential applications in medical devices and prosthetics.

Methodology

The aqueous extract of *M. titans* will be obtained following the methodology described by Peiter (2022). Borophosphate glasses will be synthesized via the melting quenching method described by (Saracini et al., 2021). The biocomposite will be evaluated for its antimicrobial activity. Characterization of the biocomposite will be performed using LC-MS chromatography. The antimicrobial activity tests will be conducted using two Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538 and *Listeria monocytogenes* ATCC 19111) and three Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 15442, and *Klebsiella pneumoniae* ATCC 700603). The antibacterial activity of the extracts will be evaluated using the disk diffusion method, following the guidelines of the Clinical & Laboratory Standards Institute (CLSI) document M02-A12 (2015), with modifications for natural products. The Minimum Inhibitory Concentration (MIC) will be determined by the microdilution method in 96-well plates, according to the CLSI document M7-A6 (2003). For the Minimum Bactericidal Concentration (MBC), after the incubation period in the microdilution test, 2 µL aliquots from each well will be inoculated onto Mueller-Hinton agar plates and incubated at 37 °C for 24 hours, as per CLSI guidelines, to identify the lowest concentration capable of eliminating the microorganisms. Different methodologies will be applied to evaluate the biocomposite's efficacy in preventing bacterial adhesion on prosthetic surfaces. The first is an adhesion test, where coated surfaces will be incubated with bacterial suspensions to assess initial adhesion by crystal violet staining. The second is a biofilm formation assay, evaluating the biocomposite's ability to inhibit biofilm development after 48-72 hours of bacterial incubation, with quantification through optical density or CFU. Additionally, direct antibacterial activity on coated surfaces will be tested by applying bacterial suspensions onto biocomposite-coated samples, followed by quantification of viable bacteria after incubation. Finally, degradation tests in simulated body fluids will assess compound release and sustained antimicrobial activity over time.

Expected Outcomes

The biocomposite developed from the aqueous extract of *M. titans* incorporated into borophosphate glasses is expected to exhibit a well-characterized chemical profile containing bioactive compounds with



significant antimicrobial potential. These compounds are anticipated to inhibit the growth of multidrug-resistant pathogens effectively. Additionally, the biocomposite is expected to prevent bacterial adhesion on prosthetic surfaces, contributing to the development of safer and more effective medical devices.

References

Arrieche, D., et al. Natural products from Chilean and Antarctic marine fungi and their biomedical relevance. *Marine Drugs*, **21**(2), 98, 2023. https://doi.org/10.3390/md21020098

Choi, J., & Kim, S. H. A genome tree of life for the fungi kingdom. *Proceedings of the National Academy of Sciences of the United States of America*, **114**, 9391–9396, 2017. https://doi.org/10.1073/pnas.1711939114

CLSI. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Sixth Edition. NCCLS document M7-A6 (ISBN 1-56238-486-4), 2003.

CLSI. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Twelfth Edition. CLSI document M02-A12. Wayne, PA: Clinical and Laboratory Standards Institute; 2015

Milhorini, S. D. S., et al. Fucogalactan from the giant mushroom Macrocybe titans inhibits melanoma cells migration. *Carbohydrate Polymers*, **190**, 50–56, 2018. https://doi.org/10.1016/j.carbpol.2018.02.063

Nielsen, J. C., et al. Global analysis of biosynthetic gene clusters reveals vast potential of secondary metabolite production in *Penicillium* species. *Nature Microbiology*, **2**, 17044, 2017. https://doi.org/10.1038/nmicrobiol.2017.44

Peiter, G. C. Atividade antimicrobiana e compostos bioativos dos extratos liofilizados do cogumelo gigante Macrocybe titans (H. E. Bigelow & Kimbr) Pegler, Lodge & Nakasone. *Tese (Doutorado)* – Universidade Federal do Paraná, Setor Palotina, 2022.

Pereira, F. C., et al. Analysis of the antifungal potential of Macrocybe titans extract against *Candida albicans*. *Future Microbiology*, **18**, 357–371, 2023. https://doi.org/10.2217/fmb-2022-0214

Saracini, J., et al. Borophosphate glasses as active agents for antimicrobial hydrogels. *International Journal of Pharmaceutics*, **644**, 123323, 2023. https://doi.org/10.1016/j.ijpharm.2023.123323

Tacconelli, E., et al. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. *World Health Organization*, **27**, 318–327, 2017. https://doi.org/10.1016/j.clinthera.2017.02.006

Vilarino, M., et al. Macrocybin, a natural mushroom triglyceride, reduces tumor growth in vitro and in vivo through caveolin-mediated interference with the actin cytoskeleton. *Molecules*, **25**(24), 6010, 2020. https://doi.org/10.3390/molecules2524601