



Abstract

Evaluation of Antifungal Activities of Actinobacterial Extracts Isolated from Deep-Sea *Laminaria ochroleuca* against Pathogenic Fungi [†]

Rita Maioto ^{1,*}, Inês Ribeiro ², Mariana Girão ², Maria F. Carvalho ^{2,3} and Ana Sampaio ¹

- Centro de Investigação e Tecnologias Agroambientais e Biológicas (CITAB), Universidade de Trás-os-Montes e Alto Douro, Quinta de Prados, 5000-801 Vila Real, Portugal; asampaio@utad.pt
- Interdisciplinary Center of Marine and Environmental Research, University of Porto, 4450-208 Matosinhos, Portugal; inesfcribeiro@gmail.com (I.R.); marianagirao1295@gmail.com (M.G.); mcarvalho@ciimar.up.pt (M.F.C.)
- School of Medicine and Biomedical Sciences, University of Porto, 4050-313 Porto, Portugal
- * Correspondence: rita.maioto@gmail.com
- † Presented at the 2nd International Electronic Conference on Antibiotics—Drugs for Superbugs: Antibiotic Discovery, Modes of Action And Mechanisms of Resistance, 15–30 Jun 2022; Available online: https://eca2022.sciforum.net/.

Keywords: *Candida*; *Aspergillus*; disc diffusion method; minimum inhibitory concentration; minimum fungicide concentration; germ tube; biofilm

Antifungal Activity from Marine Actinobacteria

Marine actinobacteria produce secondary metabolites with many biological activities of interest, including antifungals. As fungal infections have increased in the last decade, it is important to search for new compounds. The organisms from the Actinobacteria class, commonly known as actinobacteria are known for their ability to synthetize substances with broad biological activities [1].

In this work, we aimed to evaluate the antifungal activities of marine actinobacteria extracts against several pathogenic fungi.

Thirty extracts of actinobacteria isolated from marine macroalgae and deep-sea samples were screened against fungi: yeasts (*Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 22019, *Cryptococcus neoformans* PYCC 3957T, *Cryptococcus laurentii* ZY8) and molds (*Aspergillus flavus* ATCC 204304, *Aspergillus fumigatus* ATCC 204305, *Aspergillus brasiliensis* ATCC 16404). We performed the disk diffusion method (DD), following the CLSI guidelines M44-A, M38-A2 and M61 [2–4]. For the determination of the minimum inhibitory/fungicide concentration (MIC/MFC) we chose extracts with inhibition zones \geq 15 mm, the cut-off for amphotericin B. Additionally, the effect of the best extracts on biofilm and germ tube formation were studied (*Candida* spp.).

In all organisms and for DD, the susceptibilities varied with fungal species (p < 0.0001) and actinobacterial extracts (p < 0.0001). *Cr. neoformans*, and *C. albicans* were the most susceptible species. The highest MICs were obtained for *Cryptococcus* spp., *C. parapsilosis and A. flavus* (all MIC >250 µg/mL). For *A. brasiliensis*, two extracts had the lowest MICs (15.62 µg/mL). The results for *C. albicans* were in the range of 15.62–125 µg/mL, and for *C. parapsilosis* MIC was >250 µg/mL. Overall, the MFC ranged from 15.62 to >250 µg/mL. In the biofilm assay, the percentage of inhibition varied greatly between extracts (0–96%). Additionally, some extracts significantly delayed the germ tube formation in *C. albicans*.

The extracts from actinobacteria isolated from *Laminaria ochroleuca* exhibited high efficacy against fungi, and mostly against yeasts, particularly in *C. albicans* (33% of extracts), than the ones from the actinobacteria isolated from *Chondrus crispus* and *Codium tomentosum*. The dereplication analysis of the extracts explained the antifungal activity of most of them.



Citation: Maioto, R.; Ribeiro, I.; Girão, M.; Carvalho, M.F.; Sampaio, A. Evaluation of Antifungal Activities of Actinobacterial Extracts Isolated from Deep-Sea *Laminaria ochroleuca* against Pathogenic Fungi. *Med. Sci. Forum* 2022, 12, 10. https://doi.org/10.3390/eca2022-12716

Academic Editor: Marc Maresca

Published: 15 June 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

Med. Sci. Forum **2022**, 12, 10

Supplementary Materials: The following are available online at https://sciforum.net/manuscripts/12716/slides.pdf, Poster.

Author Contributions: Conceptualization, A.S. and M.F.C.; methodology, R.M., I.R., M.G. and A.S.; validation, A.S. and M.F.C.; formal analysis, R.M. and A.S.; investigation, R.M., I.R. and M.G.; resources, funding acquisition, and supervision A.S. and M.F.C.; writing—original draft preparation, R.M.; writing—review and editing, M.F.C., M.G. and A.S. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the projects "ATLANTIDA—Platform for the monitoring of the North Atlantic ocean and tools for the sustainable exploitation of the marine resources", RL4- Marine biobanks as tools for marine biotechnology NORTE-01-0145-FEDER-000040, EP1—Investigação, Desenvolvimento Tecnológico e Inovação), funded by Fundo Europeu de Desenvolvimento Regional (FEDER) through NORTE 2020, and ACTINODEEPSEA (POCI-01-0145-FEDER-031045) co-financed by COMPETE 2020, Portugal 2020 and the European Union through the European Regional Development Fund (ERDF) and by FCT, Portugal, through national funds. RM and AS, are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support by national funds FCT/MCTES to CITAB (UIDB/04033/2020) and MFC to CIIMAR (UIDB/04423/2020 and UIDP/04423/2020).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Girão, M.; Ribeiro, I.; Ribeiro, T.; Azevedo, I.C.; Pereira, F.; Urbatzka, R.; Leão, P.N.; Carvalho, M.F. Actinobacteria Isolated from *Laminaria ochroleuca*: A source of new bioactive compounds. *Front. Microbiol.* **2019**, *10*, 683. [CrossRef] [PubMed]

- 2. CLSI. CLSI Guideline M44, 3rd ed.; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2018.
- CLSI. Approved Standard—Second Edition. CLSI Document M38-A2; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2008.
- 4. CLSI. CLSI Supplement M61, 1st ed.; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2017.