

12º ENCONTRO NACIONAL

# CROMA- TOGRAFIA

6 › 8 dez'22

Aveiro | Portugal



universidade  
de aveiro



SOCIEDADE PORTUGUESA DE QUÍMICA

XIV **WARPA**  
Workshop em Análises Recentes  
no Programa de Análises

**TÍTULO:**

Livro de Resumos do 12º Encontro Nacional de Cromatografia & XIV WARPA

**AUTOR:**

Sílvia M. Rocha

**CO-AUTOR(ES):**

Alexandre Fonseca

Cátia Martins

Manuel António Coimbra

Maria Eugénia Queiroz

Samuel Patinha

Sónia Ribeiro

**EDITOR:** Sociedade Portuguesa de Química (SPQ)

**SUPORTE:** Eletrónico

**FORMATO:** PDF / PDF/A

ISBN 978-989-8124-37-1



9 789898 124371

## P82 Peptide fraction identification by SE-HPLC and LC-MS/MS analysis of the body mucus from Portugal coastal fish *Halobatrachus didactylus*

Cunha M,<sup>1</sup> Coscueta ER,<sup>1</sup> Bassesco ME,<sup>1</sup> Almada F,<sup>2</sup> Gonçalves D,<sup>3</sup> Manuela Pintado M<sup>1</sup>

1 Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005, Porto, Portugal.

2 MARE—Marine and Environmental Sciences Centre, ISPA Instituto Universitário de Ciências Psicológicas, Sociais e da Vida, Lisbon, Portugal.

3 Institute of Science and Environment, University of Saint Joseph, Rua de Londres 106, Macau S.A.R., China.

Email: mfcunha@ucp.pt

The mucus covers the fish's body, working as a protective barrier. Besides physical protection, mucus provides molecules that protect the fish from pathogens damaging <sup>1,2</sup>. These include antimicrobial peptides secreted in the mucus, which play an essential role in defense against microbial pathogens since these belong to the innate immune system<sup>2,3</sup>. In this study, two adult *Halobatrachus didactylus* individuals were captured from the wild in Sesimbra. Then, mucus collection was performed by scraping the dorsal-lateral body of the fish with a sponge. Our objective was the identification of new peptides with bioactive potential in mucus samples by chromatography analysis. Size exclusion high-performance liquid chromatography (SE-HPLC) analysis performed on mucus samples from the two individuals revealed a similar profile with an intense highlight peak which resulted in a distribution of about 775 Dalton. With interest in that peak, the two mucus samples were pooled for fractionation by SEC. The resulting fraction was analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) to identify the most probable peptide sequences. Identification from databases did not provide reliable results, indicating a lack of information on the matrix analyzed. We resorted to de novo sequencing with good results using PEAKS Studio software. Five identified peptides were selected according to their bioactivities predicted *in silico*. Furthermore, the five identified peptides were synthesized, and the molecular size was validated by SE-HPLC analysis. Overall, this chromatographic approach enabled the identification of promising peptides, which bioactivities will be evaluated *in vitro* in future work.

**Acknowledgments:** The authors acknowledge the financial support by FCT-FDCT project MACAU/0003/2019 and FDCT 0001/2020/AFJ - "FISHMUC - Bioactive properties of external mucus isolated from coastal fish of Macao and Portugal". This work was also supported by the FCT projects MARE (MAREISPA): MARE/UIDB/MAR/04292/2020 and MARE/UIDP/MAR/04292/2020.

### References

1. A. Sridhar, D. B. Manikandan, S. K. Marimuthu, T. Ramasamy, Int. J. of Peptide Research and Therapeutics 27(2) (2021) 1429-1440.
2. M. D. Fast, D. E. Sims, J. F. Burka, A. Mustafa, N. W. Ross, Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 132(3) (2002) 645-657.
3. V. Rajanbabu, J. Y. Chen, Peptides 32(2) (2011) 415-420.