

NanoLC-MS/MS sequencing and *in silico* analysis of bioactive peptides from Lusitanian Toadfish *Halobatrachus didactylus* body mucus

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Introduction

The mucus covers the fish's body, working as a protective barrier. Besides physical protection, mucus provides molecules that protect the fish from damaging pathogens [1,2]. Has been reported that antimicrobial peptides are secreted in the mucus, which play an essential role in defense against microbial pathogens since these belong to the innate immune system [2,3].



Objectives

The present study aimed to identify and characterize new peptides with bioactive potential in mucus samples by chromatography analysis.



Methodology

Sampling



For this study, we captured two adult *Halobatrachus didactylus* individuals from the wild in Sesimbra. Then, we collected mucus by scraping the dorsal-lateral body of the fish with a sponge.

Methods for Peptide Fraction analysis [4]



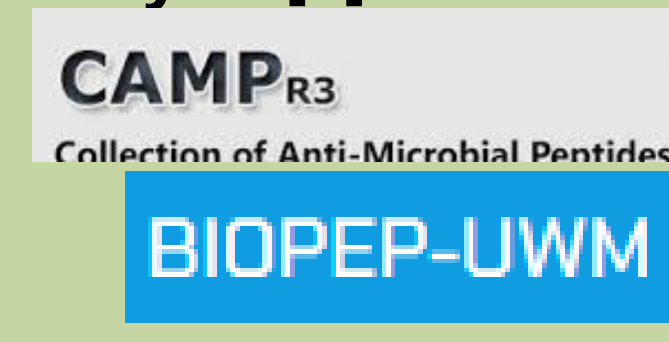
SEC Fractionation



LC-MS/MS



Proteomic Software



Bioactivity prediction



Results

Fractionation by SEC of pooled sample

The pool mucus sample from the two individuals was fractionated by size exclusion chromatography (SEC) to separate the most intense peak (Figure 1). The collected fraction was subsequently analyzed by nanoLC-MS/MS to identify the peptides.

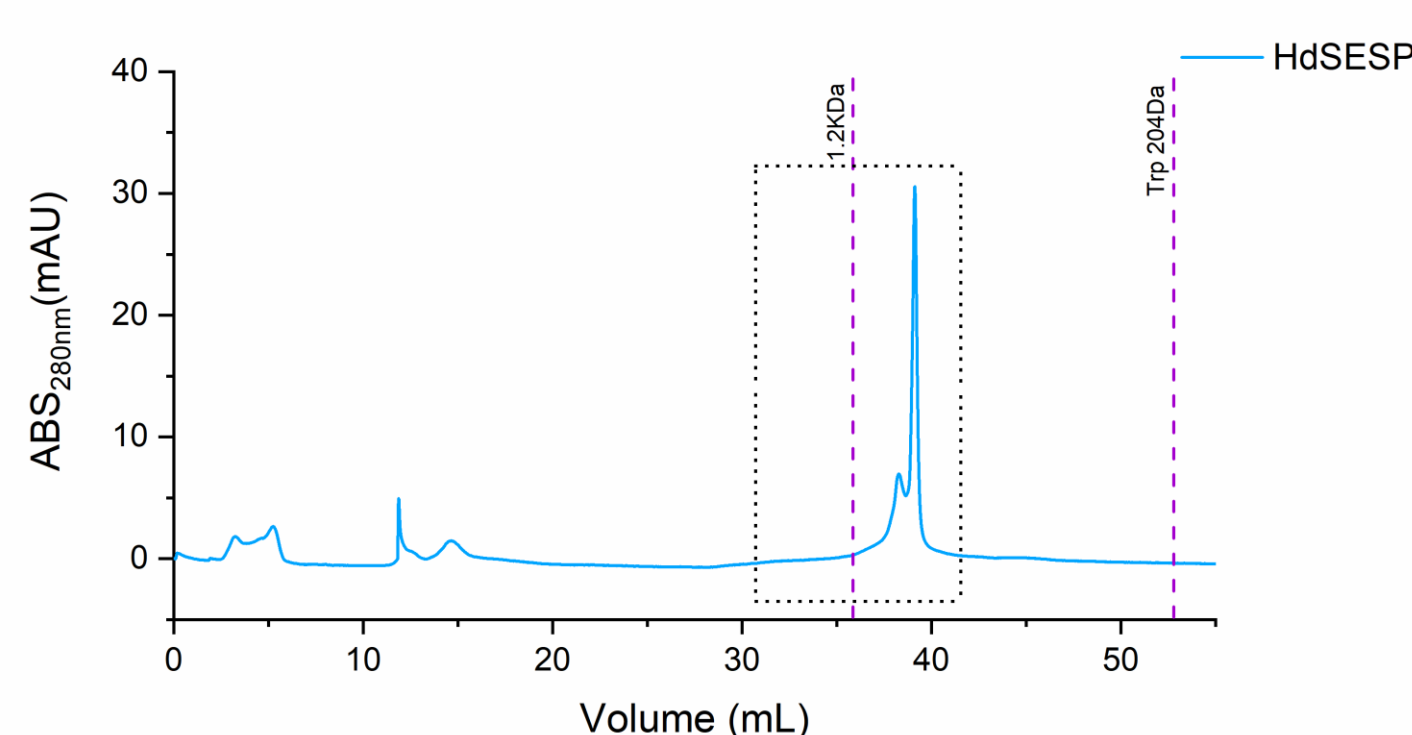


Figure 1. SEC chromatogram of the *H. didactylus* pooled sample HdSESP.

Identification of peptide fraction by nanoLC-MS/MS

Due to the lack of information in the proteomic databases for *Halobatrachus didactylus*, reliable results in peptide identification of the sample were not achieved. Therefore, it was decided to apply de novo sequencing using one of the most reliable software, PEAKS Studio®. The number of MS scans was 25686, with a number of MS/MS scans of 3329. In the analysis process, the number of De novo peptides after the score filter (De novo score ≥ 80) was 37 (Figure 2). The analysis was satisfactory in relation to the high degree of confidence of the sequencing.

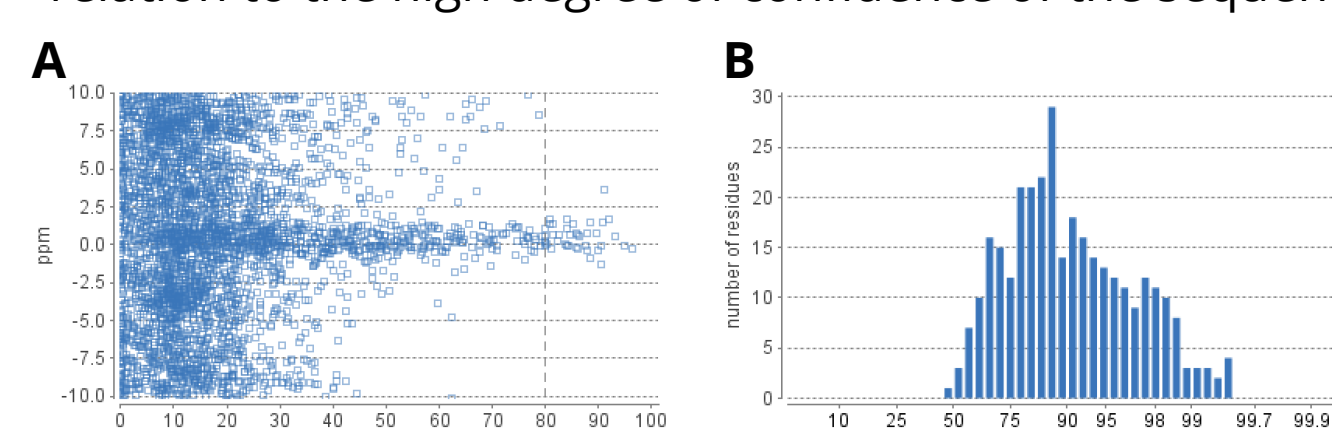


Figure 2. Denovo Statistics representation. (A) Scatterplot of peptide Denovo score versus precursor mass error. (B) Distribution of residue local confidence in the filtered result.

Five identified peptides were selected according to their bioactivities predicted *in silico* (Table 1).

Table 1. Mass spectrums of the identified peptides.

Code	MW (Da)	Sequence	Mass spectrum
HdKTLR	1588.4	EDNSELGQETPTLR	
HdKKNL	893.8	DPPNPKNL	
HdPPP	768.8	PAPPPPPP	
HdVLPN	1099.6	VYPFPGPLPN	
HdLPN	837.5	PFPGLPN	

Bioactivity prediction

CAMPR3

Table 2. Peptide classification based on antimicrobial potential predicted *in silico*.

Mass (Da)	Area	Seq. ¹	Class ^{2,3}			
			SVM	RF	ANN	DA
1589.8	2.89E+08	EDNSELGQETPTLR	AMP	NAMP	NAMP	NAMP
1101.6	1.26E+08	VYPFPGPLPN	NAMP	NAMP	AMP	NAMP
839.5	9.33E+07	PFPGLPN	NAMP	NAMP	AMP	NAMP
895.5	1.59E+07	DPPNPKNL	AMP	AMP	AMP	AMP
770.4	2.05E+06	PAPPPPPP	AMP	AMP	NAMP	NAMP

(1)The peptides are arranged in descending order of abundance, as indicated by the "Area" column. (2) AMP denotes peptides classified as potentially antimicrobial, while NAMP denotes peptides not exhibiting antimicrobial activity. (3) SVM, RF, ANN, and DA represent the classification results obtained from CAMPR3 classifiers

BIOPEP-UWM

Table 3. Peptide classification based on potential bioactivity predicted *in silico*.

Seq. ¹	Peptide Ranker ²	A ³															
		ACE inhibitor	Alpha-glucosidase inhibitor	Antianesthetic	Antioxidative	Antithrombotic	Chemotactic	Dipeptidyl peptidase III inhibitor	Dipeptidyl peptidase IV inhibitor	Immunomodulating	Inhibitor	Neuropeptide	Opioid agonist	Opioid	Regulating	Renin inhibitor	Stimulating
DPPNPKNL	0.73075	0.125	0.125						0.750								
PAPPPPPP	0.96263	1.375	0.625						1.250								
EDNSELGQETPTLR	0.04606	0.357		0.071				0.071	0.429		0.071				0.071	0.071	
VYPFPGPLPN	0.96638	1.200	0.100	0.300	0.100	0.300	0.100	0.200	0.900	0.200	0.100		0.100	0.200	0.300		
PFPGLPN	0.91394	1.000		0.375		0.375	0.125	0.125	0.875		0.125				0.375		

(1) The peptides are arranged in descending order based on their potential antimicrobial prediction by CAMPR3, with secondary order given to their abundance. (2) PeptideRanker scores indicate the probability of the peptide being bioactive. (3) The "A" represents the frequency of bioactive fragments in a protein, categorized by various bioactivity types predicted by BIOPEP-UWM.

Conclusions

Overall, this chromatographic approach enabled the identification of promising peptides, while the bioinformatic tools facilitated the prediction of their bioactivity. This is a crucial step for drug development, as it streamlines discovery, reduces costs, and accelerates the identification of new and effective healthcare outcomes.

Acknowledgements

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References

- A. Sridhar, D. B. Manikandan, S. K. Marimuthu, T. Ramasamy, Int. J. of Peptide Research and Therapeutics 27(2) (2021) 1429-1440.
- 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.
- V. Rajanbabu, J. Y. Chen, Peptides 32(2) (2011) 415-420.
- Fernandez Cunha, M., Coscueta, E. R., Brassesco, M. E., Marques, R., Neto, J., Almada, F., Gonçalves, D., and Pintado, M, Molecules, 28(18) (2023) 6458.