

Bioactive Potential and Chromatographic Characterization of Body Mucus from Portugal Coastal Fish *Halobatrachus didactylus*

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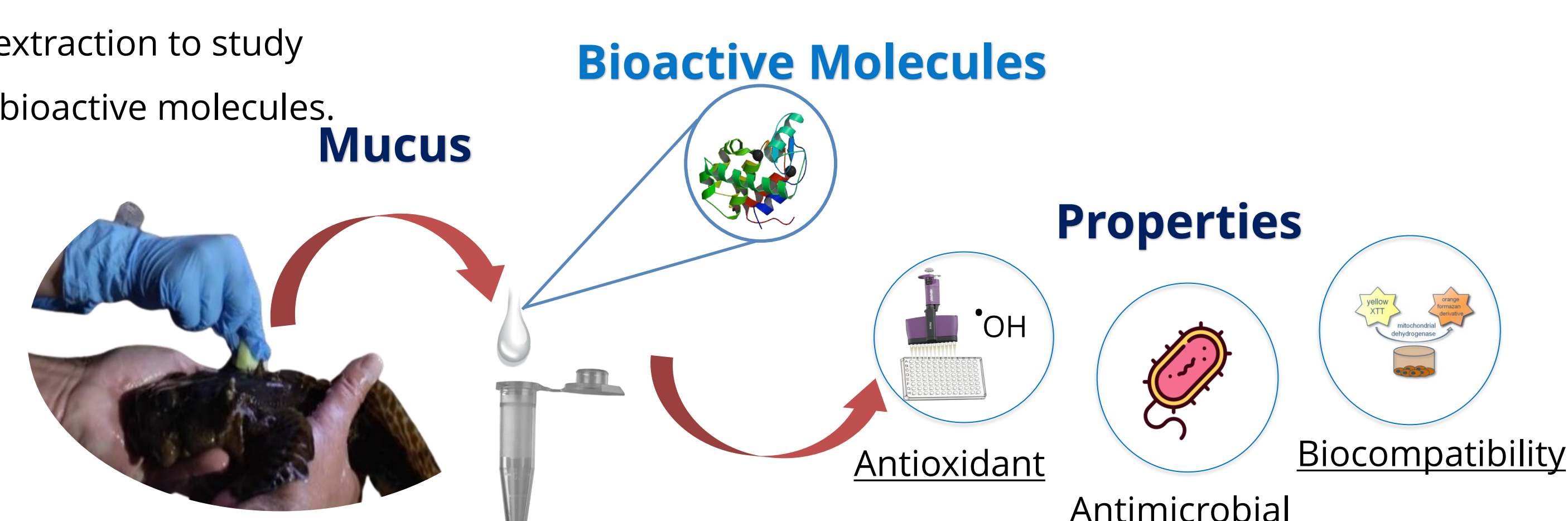


Introduction

Fish body mucus and its components provide the first line of defense as a stable physical or chemical barrier against invading pathogens. Some of these components are antimicrobial peptides secreted as a response to immune stimulation. Some studies have shown antimicrobial activity against multidrug resistant pathogens and low toxicity to eukaryotic cells [1]. Until now, the mucus of *H. didactylus* had not been studied, a species that is reportedly toxic to other fish species. Marine fish may represent an untapped source of bioactive molecules for potential applications in health promotion and the food industry.

Objectives

Mucus extraction to study potential bioactive molecules.



Methods

Mucus collection

- Five *Halobatrachus didactylus* individuals from the Aquarium Vasco da Gama in Lisbon (HdAVG).
- Scraping the dorsal-lateral body of the fish with a sponge.

SE-HPLC

- Agilent AdvanceBio SEC column 2.7 μ m particle size, 130 Å pore size, and 7.8 inner diameter x 300 mm length.

Biochemical assays

- Protein quantification (BCA).
- ABTS scavenging assay adapted from [2].
- ORAC assay adapted from [3].

Antimicrobial

- Inhibition growth curves from [4].

Biocompatibility

- HaCaT cells
- Caco2 cells
- Presto Blue cell viability assay from [5,6].

Results

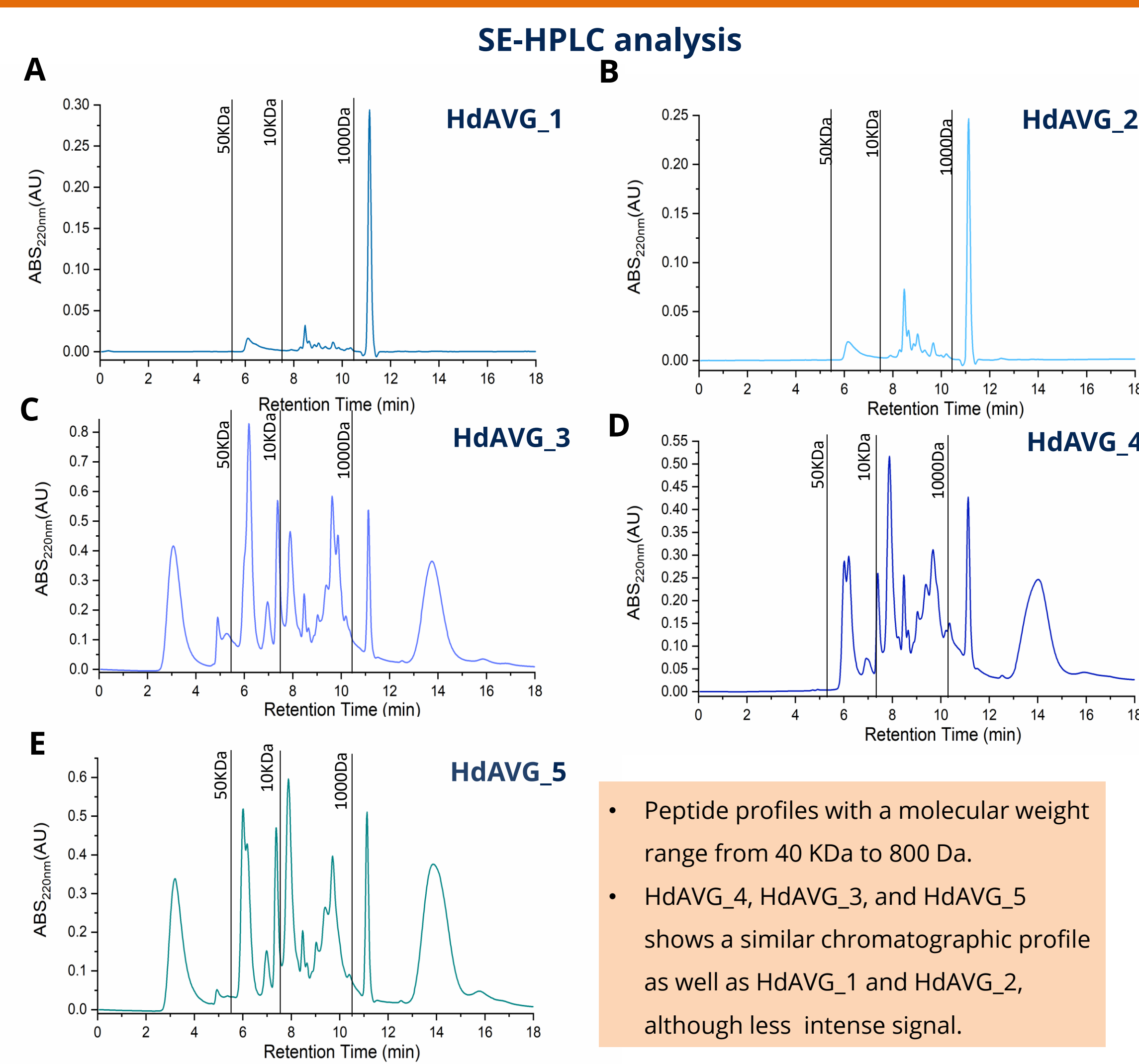


Figure 1. SE-HPLC chromatograms of mucus samples from the five individuals *H. didactylus* from Aquarium Vasco da Gama (HdAVG): (A) HdAVG_1, (B) HdAVG_2, (C) HdAVG_3, (D) HdAVG_4 and (E) HdAVG_5.

Pool (HdAVG_1+HdAVG_2+HdAVG_3+HdAVG_4+HdAVG_5) of the mucus samples to obtain an adequately concentrated mucus to carry out the **bioactivities**.

Total Protein and Antioxidant activity

- The pooled mucus sample showed a significant concentration of total protein which is consistent with antioxidant potential (ABTS and ORAC).

Table 1. Total protein quantification (BCA) and antioxidant activity (ABTS and ORAC) of pooled mucus sample from *H. didactylus* individuals.

Total protein	16836 \pm 1020 μ g BSA/mL
ABTS	268 \pm 11 μ mol TE/g mucus protein
ORAC	306 \pm 11 μ mol TE/g mucus protein

Biocompatibility

- The pooled mucus sample was biocompatible for Caco-2 cells in concentrations between 196 to 25 μ g mucus protein/mL, while it was toxic for HaCaT cells.

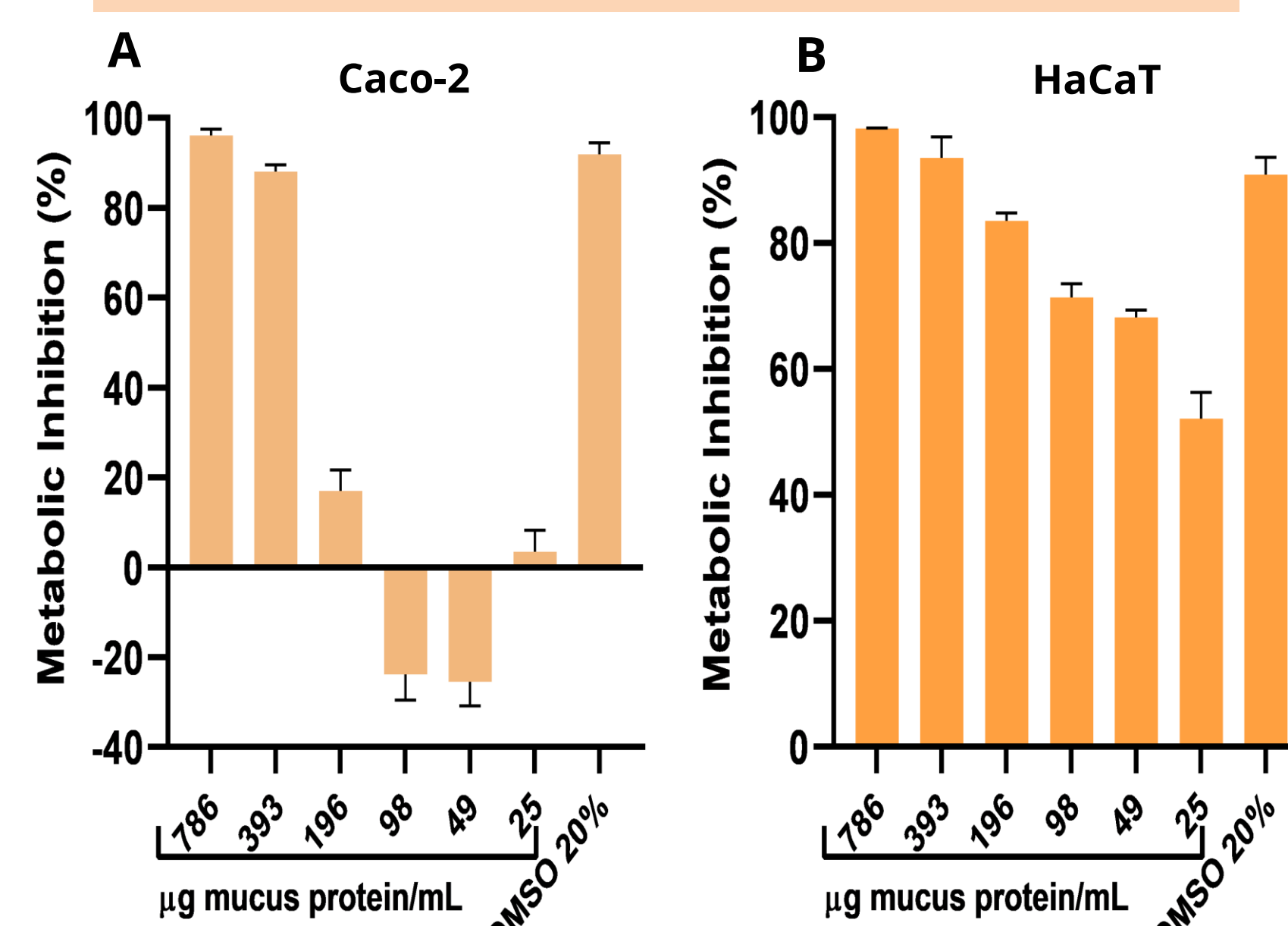
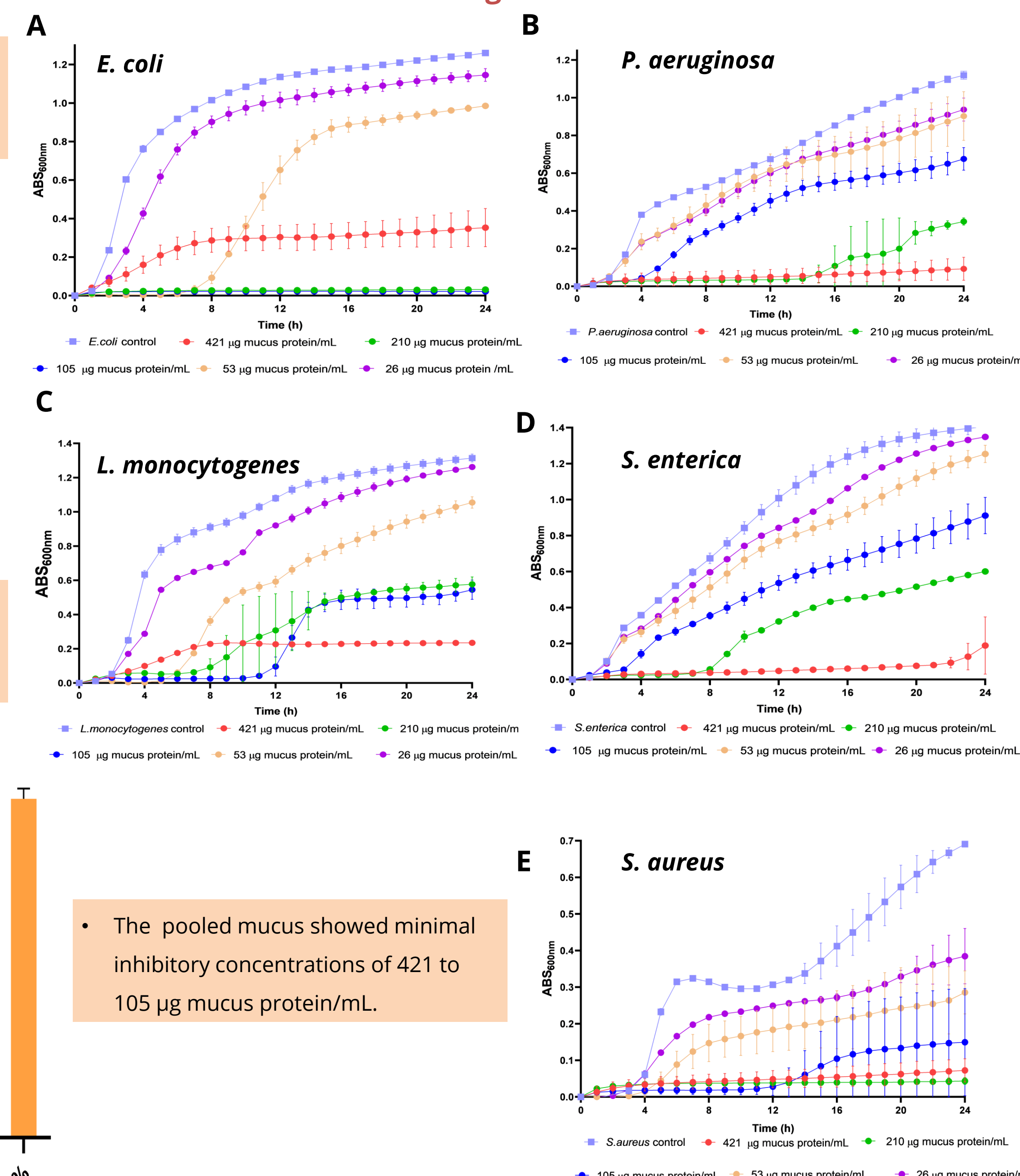


Figure 2. Biocompatibility of mucus sample on (A) Caco-2 and (B) HaCaT.

Inhibition growth curves



- The pooled mucus showed minimal inhibitory concentrations of 421 to 105 μ g mucus protein/mL.

Figure 3. Inhibition growth curves of mucus sample tested at concentrations between 421 to 26 μ g mucus protein/mL against pathogenic bacteria: (A) *E. coli*, (B) *P. aeruginosa*, (C) *L. monocytogenes*, (D) *S. enterica*, and (E) *S. aureus*.

Conclusions

Overall, the body mucus sample from *H. didactylus* showed the potentiality of having peptides with antimicrobial and antioxidant properties with the possibility of future application in the health and food industry. These promising results were obtained from a single species reportedly producing ichthyocirrinotoxins that affect other fish. Considering that there are more than 2000 toxic/venomous fish species whose bioactive compounds were not yet evaluated, this group of vertebrates may represent a promising field of research for blue biotechnology.

Acknowledgements

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