

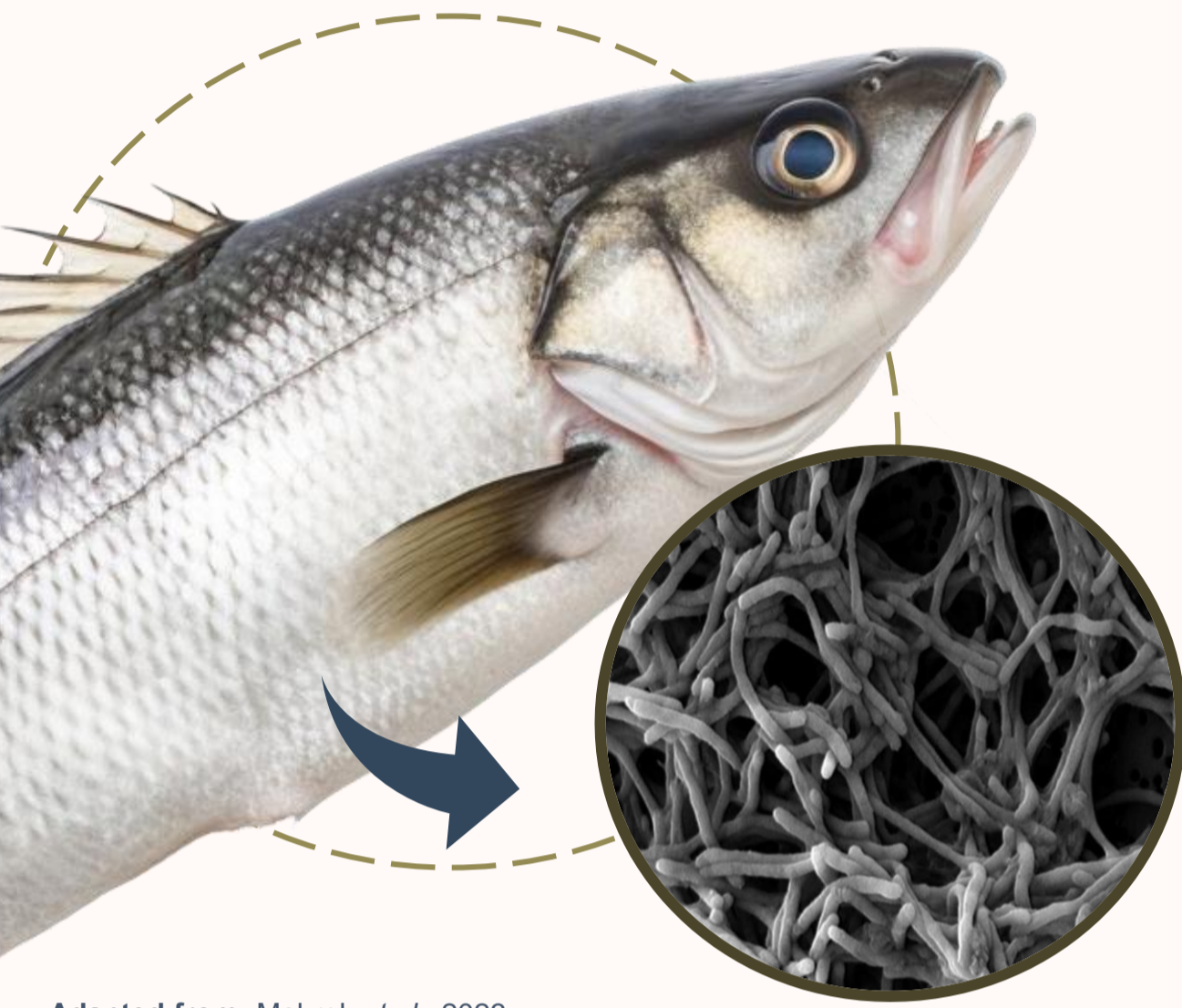
# IDENTIFICATION OF *TENACIBACULUM MARITIMUM* EXTRACELLULAR PRODUCTS: *IN VITRO* AND *IN VIVO* PROTEOMIC APPROACH

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## INTRODUCTION

- *Tenacibaculum maritimum* has been a cause for concern due to re-emergent global outbreaks in aquaculture sites
- Extracellular products (ECPs) secreted by *T. maritimum* are one of its main virulence mechanisms

(Escribano et al., 2024; Mabrok et al., 2023)



Adapted from: Mabrok et al., 2023

## HOWEVER...

ECPs remain largely unexplored, both *in vitro* and *in vivo*

**AIM** → Identification of proteins secreted *in vitro* and *in vivo* by a *T. maritimum* virulent strain

## MATERIAL & METHODS

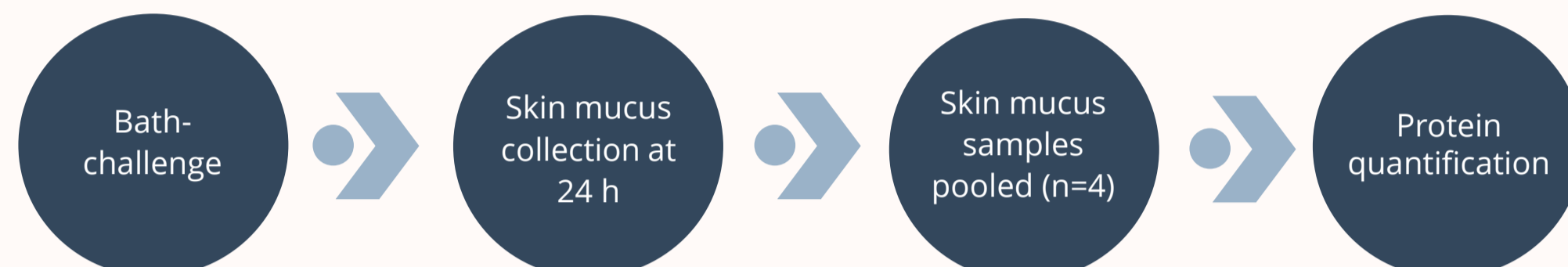
*T. maritimum* (ACC13.1) was used in all experiments:

### *In vitro* experiment



### *In vivo* experiment

Bath-challenge:  $5 \times 10^5$  CFU mL<sup>-1</sup> *T. maritimum*



European seabass juveniles (45.5 ± 8.1 g)

NanoLC-MS/MS

Protein identification

Gene Ontology (GO) enrichment analysis: UniProt ID mapping platform

## RESULTS & DISCUSSION

### *IN VITRO* EXPERIMENT

Band sizes ranging from over 120 kDa to less than 12 kDa

- Excised protein bands identified as B1, B2, B3, B4 and B5

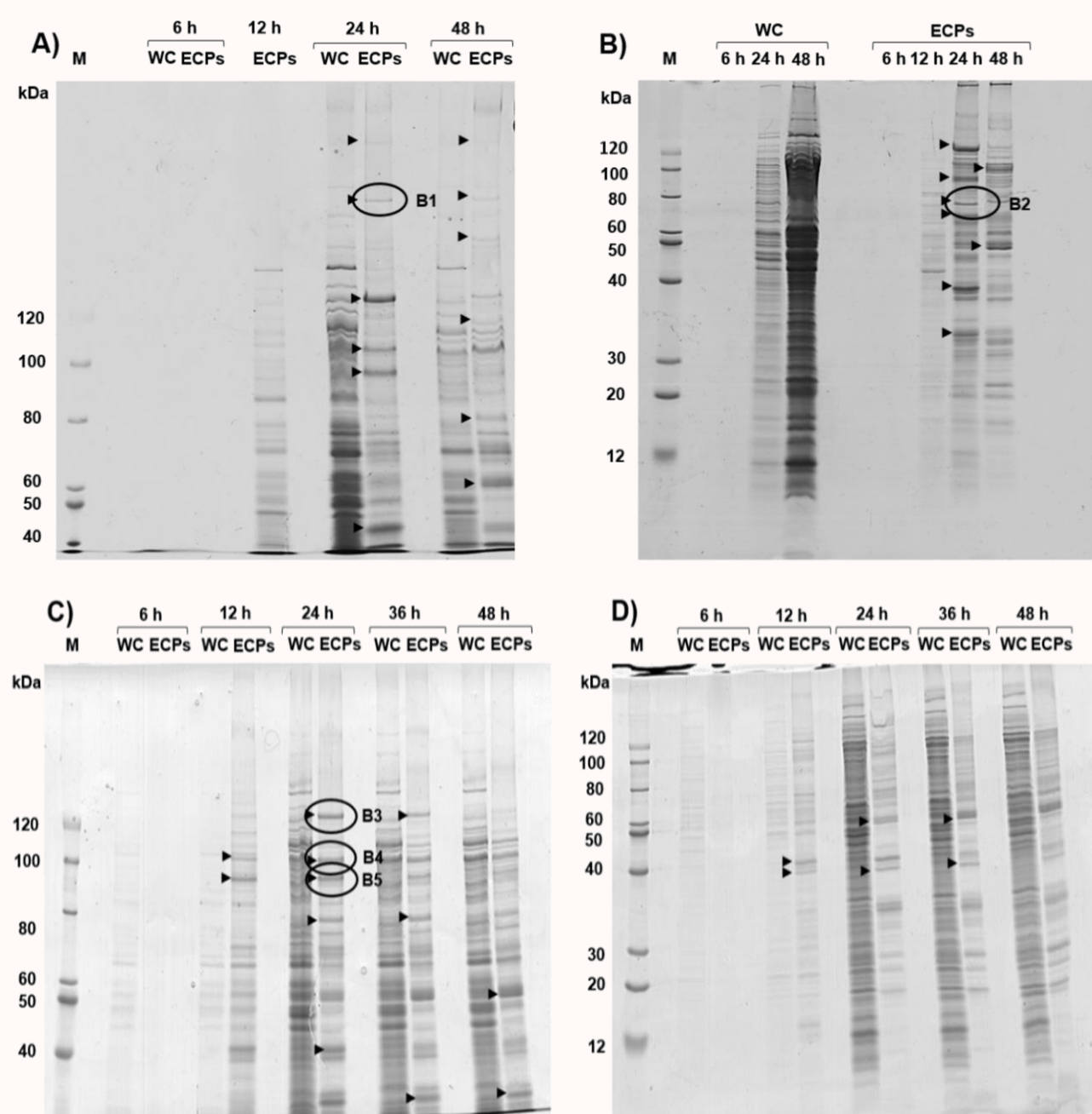


Fig. 1: SDS-PAGE analysis of whole cells (WC) and cell-free culture supernatants (ECPs) of *T. maritimum* (strain ACC13.1) for experiments 1 (A and B) and 2 (C and D). Analysis was performed in an 8% (A and C) or 14% (B and D) polyacrylamide gel. Each lane contains protein equivalent to 1 mL of bacterial culture. The gels were stained with Coomassie Brilliant Blue. M-molecular weight marker (GRS Unstained Protein Marker, GRISP); numbers on the left indicate the molecular weight of the markers in kDa. Arrowheads indicate putative secreted proteins (in ECPs, not WC). The excised bands analyzed by NanoLC-MS/MS are marked with a circle.

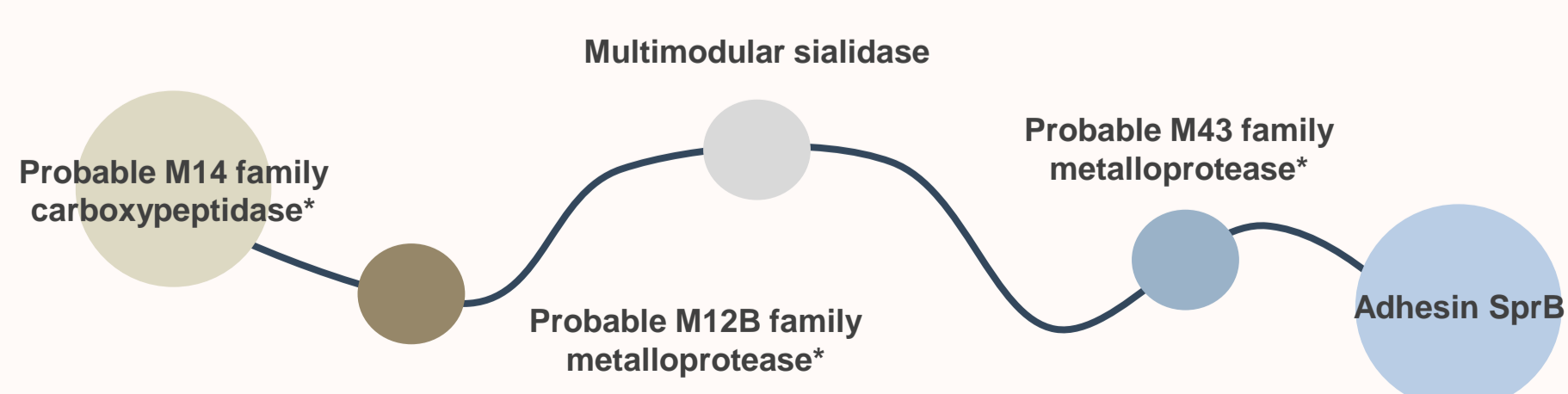
### Protein identification:

Top 3 most abundant proteins for each excised band

B1	Protein ID	Abundance (%)	B2	Protein ID	Abundance (%)	B3	Protein ID	Abundance (%)
➤	Multimodular sialidase/Sialate *	89.56	➤	Gliding motility lipoprotein GldJ	46.75	➤	Probable M14 family carboxypeptidase *	95.8
➤	Probable M14 family carboxypeptidase *	4.72	➤	Succinate dehydrogenase	6.83	➤	Endonuclease *	0.82
➤	Adhesin SprB	2.25	➤	Probable M12B family metalloprotease *	5.89	➤	Uncharacterized protein	0.48
B4	Protein ID	Abundance (%)	B5	Protein ID	Abundance (%)			
➤	Probable M14 family carboxypeptidase *	55.20	➤	Probable M43 family metalloprotease *	89.75			
➤	Uncharacterized protein	31.94	➤	Probable M14 family carboxypeptidase *	2.45			
➤	TonB-dependent outer membrane receptor	2.66	➤	Probable M12B family metalloprotease *	1.90			

\* C-terminal secretion signal

Several common proteins among excised bands...



**Acknowledgments**  
Work funded by the European Union's Horizon Europe research and innovation program (GA No. 101084651 - project IGNITION)

### References

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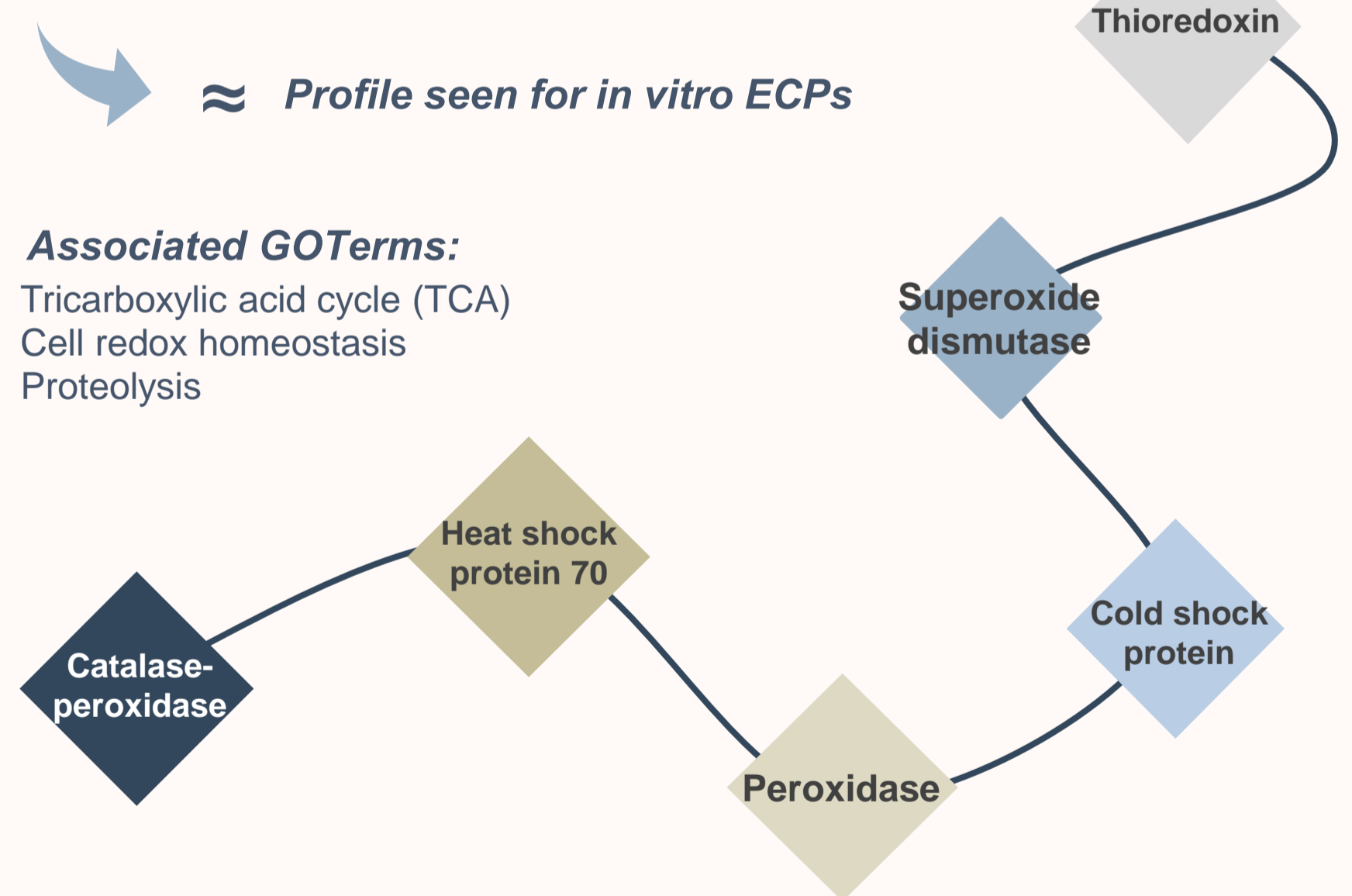
### *IN VIVO* EXPERIMENT

- 32.1% mortality in bath-challenge fish
- Clinical symptoms matching tenacibaculosis outbreak

### Protein identification:

- Probable M43 family metalloprotease (C-terminal secretion signal)
- Probable M12B family metalloprotease (C-terminal secretion signal)
- Exo-alpha-sialidase
- Adhesin SprB
- Lipoproteins

Plus...



**Associated GO Terms:**  
Tricarboxylic acid cycle (TCA)  
Cell redox homeostasis  
Proteolysis

Some proteins with a conserved C-terminal domain (CTD)



Indicating these can be

**Secreted virulence factors (Type IX secretion system)**

(Pérez-Pascual et al., 2017)

## TAKE-HOME MESSAGES

***In vitro*:** Presence of a complex protein profile (several proteins potentially important for virulence)

***In vivo*:** Identification of proteins related to resistance against oxidative stress and phagocytosis

**M12B + M43 metalloproteases are present in the skin mucus of challenged fish**