

## Supplementary Material

### Identification and antimicrobial activity of medium-sized and short peptides from yellowfin tuna (*Thunnus albacares*) simulated gastrointestinal digestion

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The ATCC bacterial strains are stored inside Microbank™ at -20 °C:

1. Defrost at room temperature, paying particular attention to the safety standards provided by class 2 laboratories
2. With a sterile loop, take a small sphere from the microbank and perform the microbiological smear on blood agar plates and incubate at 37 ° for 24 hours \*
3. Remove bacteria colonies from the bacteria plate and inoculate the tube containing the Mueller-Hinton broth 10 ml, maintain this bacterial stock culture exponentially, incubating for about 4 hours at 37 °
4. Use a densitometer McFarland DEN-1 ( 0.3 - 15.0 unit ) for obtained 0.5 unit of McFarland that corresponds roughly to  $10^8$  bacteria/mL , or It is possible use a McFarland standard that corresponds to a suspension of BaC12 prepared in H<sub>2</sub>S04 [0.05 mL of 1% BaC12 (w/v) in 9.95 mL of 10% H<sub>2</sub>S04 (v/v)] and compare the inoculum visually
5. Adjust the suspension to  $10^3$  bacteria/mL (a dilution to 1 : 10)

In order to verify the antimicrobial activity of the two extracts against the *Staphylococcus aureus* bacteria strain, two antibiotics were used as controls: Ampicillin (25 µg/mL) and Polymyxin B (400µg/mL). Data are shown in Table

Antibiotics and extracts	Bacteria strain	UFC/mL inoculo	MIC mg ml <sup>-1</sup>	UFC 24 h incubation	MCB mg ml <sup>-1</sup>
Ampicillin	<i>S.aureus</i>	1,5 x 10 <sup>3</sup>	1.5 X 10 <sup>-6</sup>	1,0 x 10 <sup>-1</sup>	1.5 X 10 <sup>-6</sup>
Polymixin B			2,4 x 10 <sup>-5</sup>	3.7 x 10 <sup>-2</sup>	2,4 x 10 <sup>-5</sup>
C18			10,0 x 10 <sup>-1</sup>	0	10,0 x 10 <sup>-1</sup>
GCB			35 x 10 <sup>-1</sup>	0	35 x 10 <sup>-1</sup>