Bacillus spp. Natural Antimicrobial Compounds are promising antimicrobial agents for aquaculture

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Introduction

Fish Diseases

One-Health	Economic losses
700,000	6 Billion USD
People die each year due to drug-resistant bacteria	Predicted annual losses due to fish diseases

Bacillus spp. as an aquaculture friendly microbe

- Antimicrobial activity
- Anti-Biofilm activity
- Quorum-Quenching activity
- Immunostimulatory activity

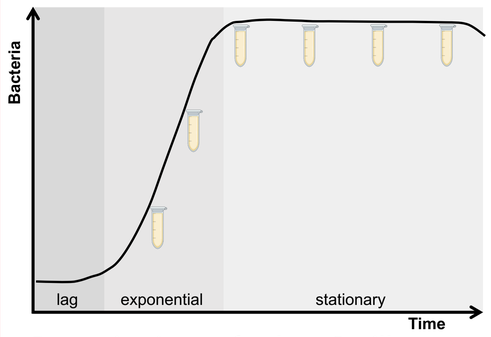
Objective

Isolate and purify the extracellular Natural Antimicrobial Compounds produced by a bioactive *Bacillus* sp. Strain.

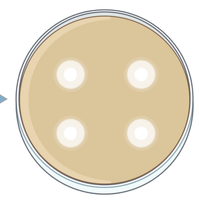
Bacillus sp. strain FI314 was previously selected based on its *in vitro* and *in vivo* bioactivities

Materials and Methods

Establishment of bioactive NACs kinetics

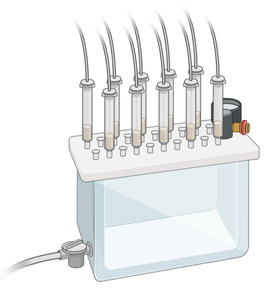


Determination of the *Bacillus* sp. growth curve and sampling of extracellular cell-free supernatant (CFS) along the growth curve

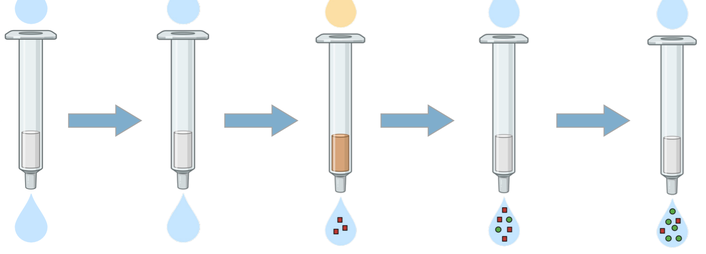


Test CFS against a broad range of bacterial fish pathogens using the well-diffusion assay

Bacillus spp. NACs purification steps



Purification of the bioactive compounds using a C18 Solid-phase extraction cartridge in a vacuum manifold



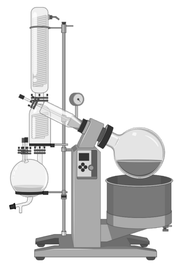
Column activation with 100% methanol

Column equilibration with methanol:H_2O (50:50)

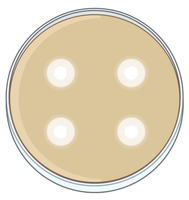
CFS loading with 5% methanol

Column wash with methanol:H_2O (50:50)

Compounds' elution with 100% methanol



Evaporation of the eluted fractions using a rotary evaporator, nitrogen and high vacuum



Test eluted fractions (50 mg mL^{-1} of DMSO) against bacterial fish pathogens using the well-diffusion assay

Results

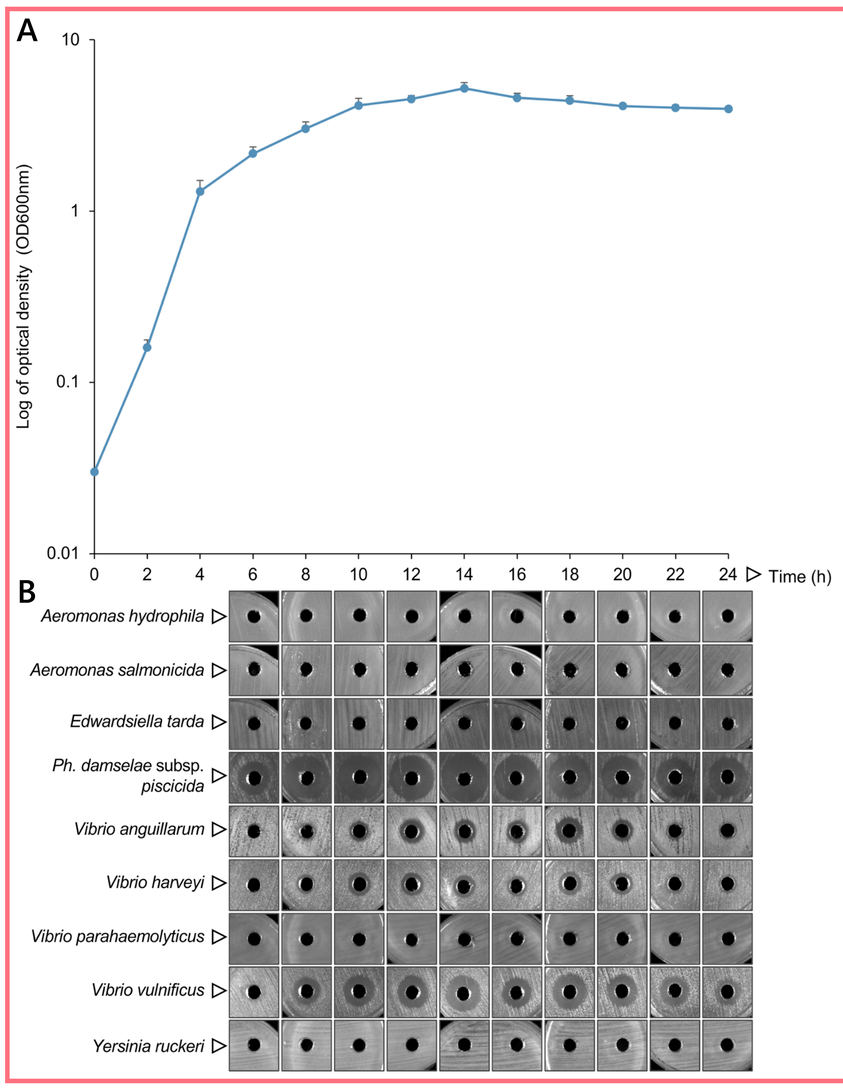


Fig 1. Growth curves and anti-growth activity kinetics of *Bacillus subtilis* FI314. (A) *B. subtilis* was grown in Luria-Bertani medium for 24h at 37ºC, 140 rpm. (B) Growth inhibition zones for the fish pathogens *A. hydrophila*, *A. salmonicida*, *E. tarda*, *Ph. damselae* subsp. *piscicida*, *V. anguillarum*, *V. harveyi*, *V. parahaemolyticus*, *V. vulnificus* and *Y. ruckeri* around the wells filled with the cell-free supernatant of *B. subtilis* FI314 previously filtered with 0.22 µm cellulose acetate filter.
All photos were taken in a Gel-Doc™ XR+System, using the Image Lab™ Software (Bio-Rad, EU) and are at the same scale.

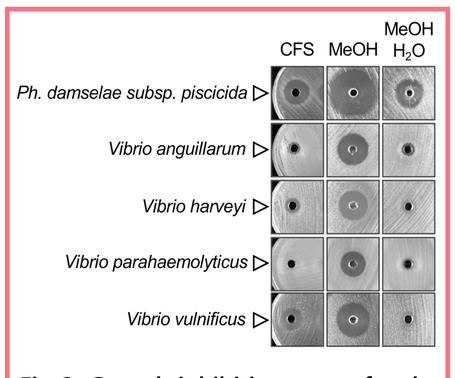


Fig 2. Growth inhibition zones for the fish pathogens *Ph. damselae* subsp. *piscicida*, *V. anguillarum*, *V. harveyi*, *V. parahaemolyticus and V. vulnificus* around the wells filled with *B. subtilis* cell-free supernatant (CFS) and the eluted fractions from the purification steps (MeoH and MeoH:H_2O). The bioactive compounds were eluted using methanol: water (50:50 – MeoH:H_2O) and 100% methanol (MeoH) as gradients. After evaporation using a rotary evaporator followed by nitrogen and high vacuum, the fractions were dissolved in DMSO to reach a final concentration of 50 mg mL^{-1}. After sterilization through filtration, 100 µL of each fraction was used to test their bioactivity.
All photos were taken in a Gel-Doc™ XR+System, using the Image Lab™ Software (Bio-Rad, EU) and are at the same scale.

Conclusions

The extracellular Natural Antimicrobial Compounds of *B. subtilis* FI314 produced clear inhibition halos against several *Vibrio* species and *Ph. damselae*

The maximum production of extracellular Natural Antimicrobial Compounds was observed during the stationary growth phase (~14-18h)

The fraction eluted with 100% methanol retained the antimicrobial activity and is currently being analysed by liquid chromatography with tandem mass spectrometry (LCMS/MS) to assess the potential novelty of its compounds

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