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SECRETARÍA DE EDUCACIÓN SUPERIOR, CIENCIA, TECNOLOGÍA E INNOVACIÓN

1er-CIQ-Ec-18
1er Congreso Internacional de Quimica Ecuador 2018
31 de julio al 2 de agosto, Riobamba

Metobolome analysis in the microbial antagonism by liquid chromatography coupled with chemometrics algorithms

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ESCUELA SUPERIOR POLITECNICA DEL LITORAL ESPOL

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31/07/2018
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Outline

- Introduction
- Objectives
- Methodology
- Results & Discussion
- Conclusion

Vibrio harveyi

marine gram-negative bacteria

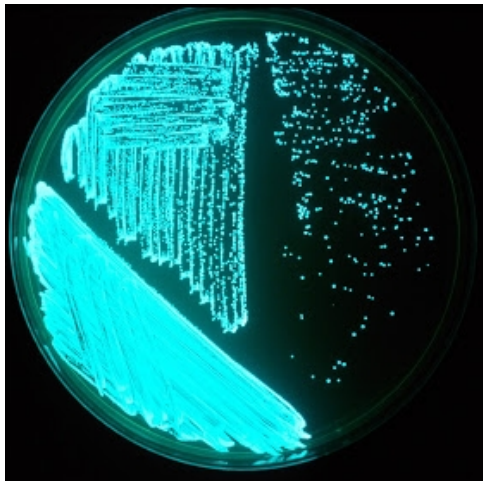

Figure. *V. harveyi* culture

Letters in Applied Microbiology

UNDER THE MICROSCOPE

Vibrio harveyi: a significant pathogen of marine vertebrates and invertebrates

B. Austin[1] and X-H. Zhang[2]

1 School of Life Sciences, John Muir Building, Heriot-Watt University, Riccarton, Edinburgh, UK
2 Department of Marine Biology, Ocean University of China, Qingdao, China

[1] Austin, B., & Zhang, X. (2006). Vibrio harveyi: a significant pathogen of marine vertebrates and invertebrates. *Letters in Applied Microbiology*, *43*(2), 119-124. doi:10.1111/j.1472-765x.2006.01989.x

Economic importance

ELSEVIER

Aquaculture 164 (1998) 337–349

Aquaculture

Mortalities of pond-cultured juvenile shrimp, *Penaeus monodon*, associated with dominance of luminescent *vibrios* in the rearing environment

C.R. Lavilla-Pitogo *, E.M. Leaño, M.G. Paner

Aquaculture Department, Southeast Asian Fisheries Development Center, Tigbauan 5021, Iloilo, Philippines

Year	Culture period (days)	Stocking density (shrimp/m^2)	Survival (%)
1994	174 (114–212)	40.3 (30–52)	29.3 (16–43)
1993	220 (169–260)	28.0 (20–33)	63.0 (48–79)
1992	174 (152–227)	20.0 (15–25)	86.6 (80–94)

Yearly average of farm efficiency of *P. monodon* in vibriosis episodes [2].

[2] Lavilla-Pitogo, C., Leaño, E., & Paner, M. (1998). Mortalities of pond-cultured juvenile shrimp, Penaeus monodon, associated with dominance of luminescent vibrios in the rearing environment. *Aquaculture*, *164*(1-4), 337-349. doi:10.1016/s0044-8486(98)00198-7

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Pseudovibrio denitrificans

Introduction

Bacteria marina gram negativa perteneciente a la clase α-protobacteria

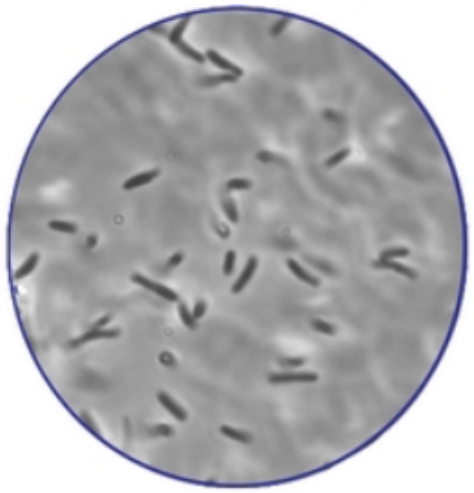

Figura. Microscopía óptica de *P. denitrificans*

Mar. Drugs **2014**, *12*, 5916-5929; doi:10.3390/md12125916

OPEN ACCESS

marine drugs

ISSN 1660-3397

www.mdpi.com/journal/marinedrugs

Review

Marine *Pseudovibrio* sp. as a Novel Source of Antimicrobials

Susan P. Crowley [1,2], Fergal O'Gara [2,3], Orla O'Sullivan [1,4], Paul D. Cotter [1,4,*] and Alan D. W. Dobson [2,5]

[3] Crowley, S., O'Gara, F., O'Sullivan, O., Cotter, P., & Dobson, A. (2014). Marine Pseudovibrio sp. as a Novel Source of Antimicrobials. *Marine Drugs*, *12*(12), 5916-5929. doi:10.3390/md12125916

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Objectives

Objectives

General objective

To analyze microbial antagonism metabolome between *Pseudovibrio denitrificans* and *Vibrio harveyi* to identify possible biomolecules with biological activity

Specific objectives

- To use liquid chromatography obtaining metabolomic profiles
- To analyze chromatograms with multivariate chemometrics algorithms

Microorganisms

Metodología

Reserva Marina
El Pelado

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Microorganisms culture

Methodology

V. harveyi (V.h)

Microbial culture collection

P. denitrificans (P.d)

Isolated from the Marine reserve

Cultured by 48H

Antagonism Interaction 72H

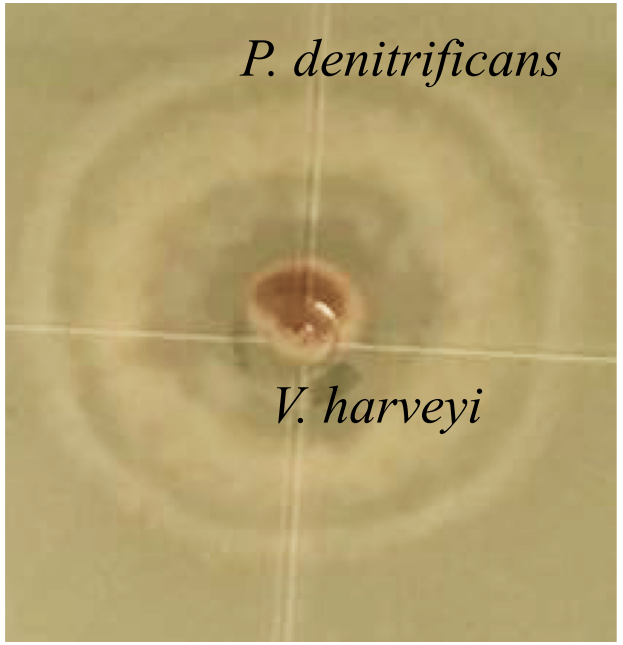

Figure. Antagonism interaction after 72H

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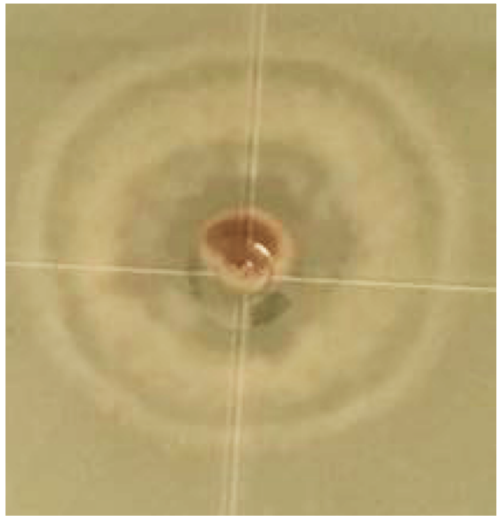
Sample preparation

Methodology

Petri Dish → Culture media/ Bacterial colonies → Liquid-Liquid extraction (Ethyl acetate) → Solid Phase Extraction (C_{18}, 5 Eluents)



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Liquid Chromatography Data Analysis

Methodology

- Automatic injection
- UHPLC-DAD (Waters)
- Reverse phase T3 column (C_{18})
- 50 x 2.1 mm y de 1,7 µm particle size
- Absorbance 254 nm

Preprocesing

- Noise reduction
- Baseline correction

Multivariate chemometrics

- PCA (Scaled & mean centered)

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Experimental Design

Methodology

Sample matrix

1. Culture media
2. Bacterial colonies

(2 levels)

Sample type

1. V. haveryi
2. P. denitrificans
3. Antagonism
4. Control

(4 levels)

SPE eluent

- F2: H_2O:MeOH 1:1
- F3: MeOH 100%
- F4: MeOH:CH_2CL_2

(3 levels)

2 * 4 * 3 = 36 (conditions)
36 * 3 = 72 (experiments)

Chromatograms (Sample Matrix)

Results & Discussion

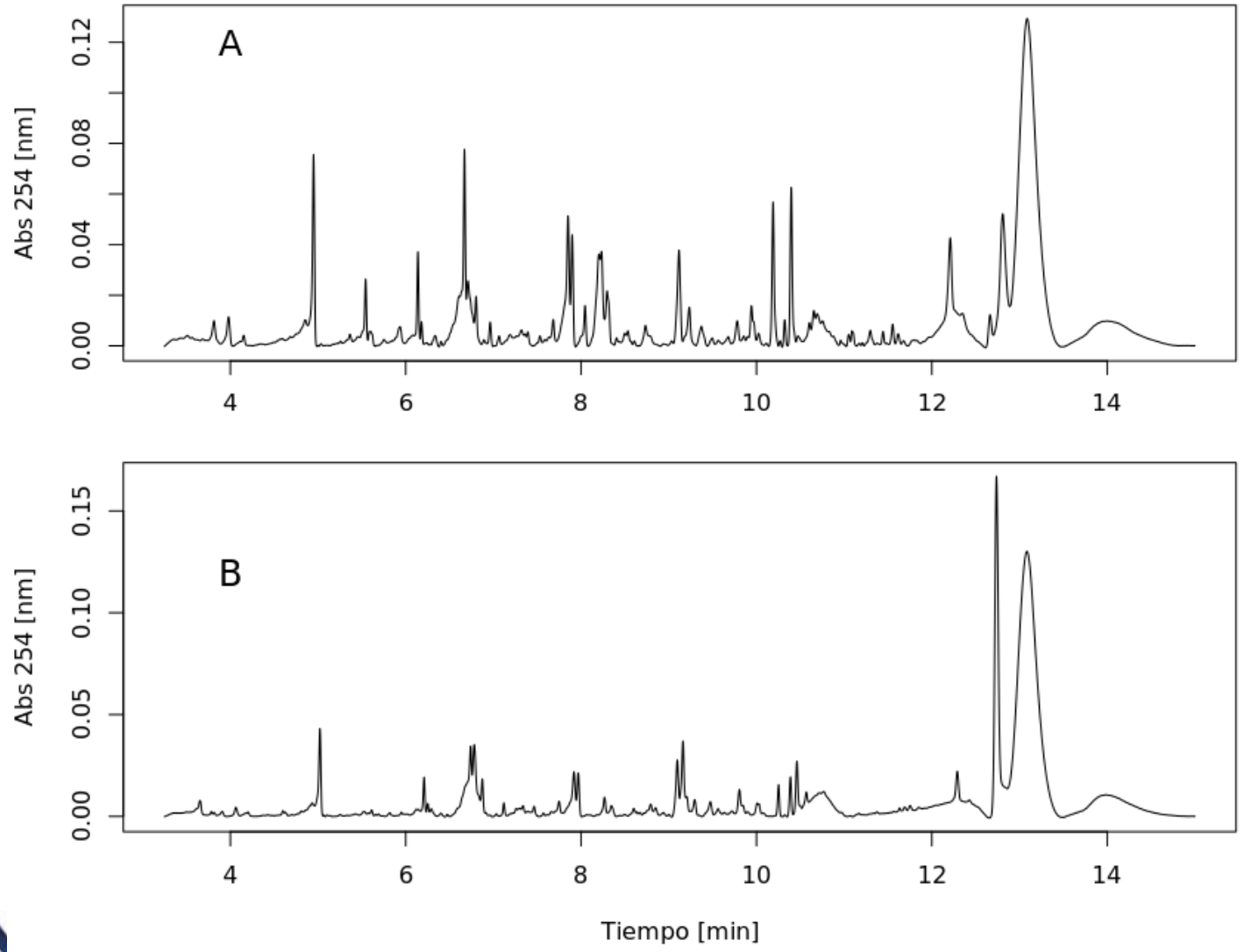

Figure. Representative chromatograms of antagonism interaction. (A) culture media and (B) bacterial colonies

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PCA (Sample Matrix)

Results & Discussion

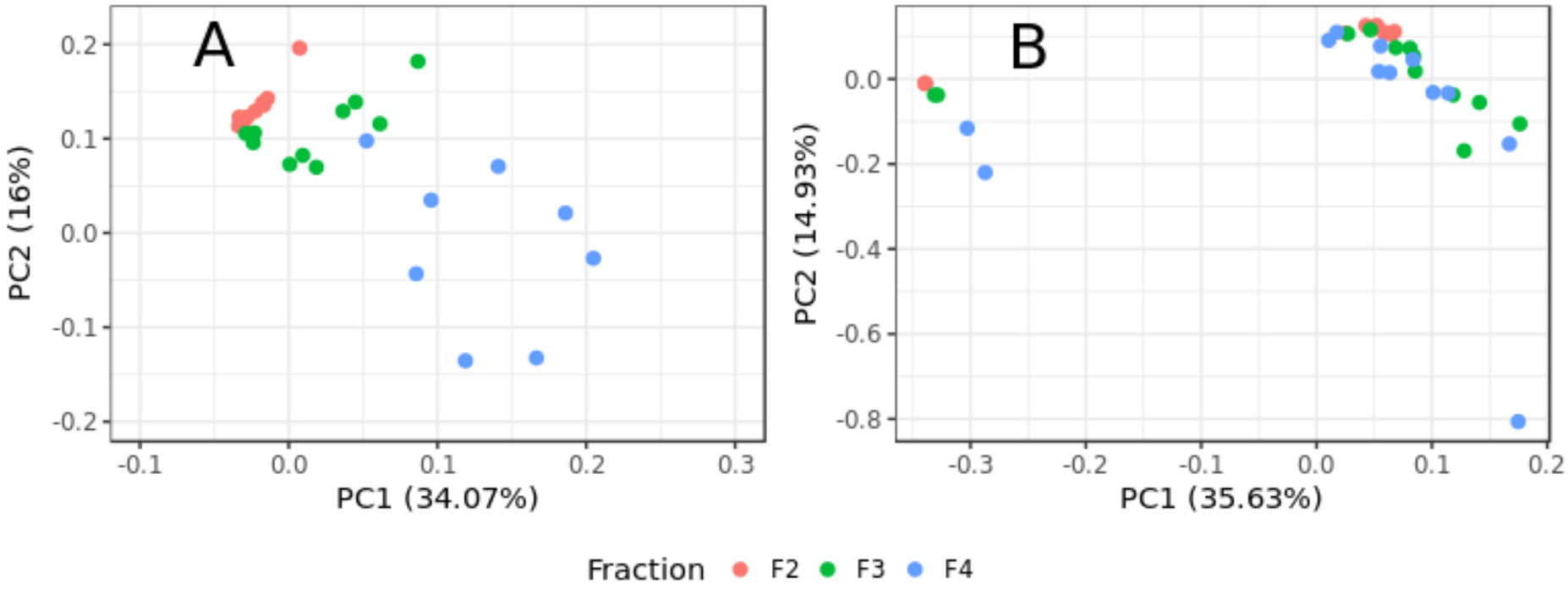

Figure. (A) culture media and (B) bacterial colonies

PCA (Sample Type)

Results & Discussion

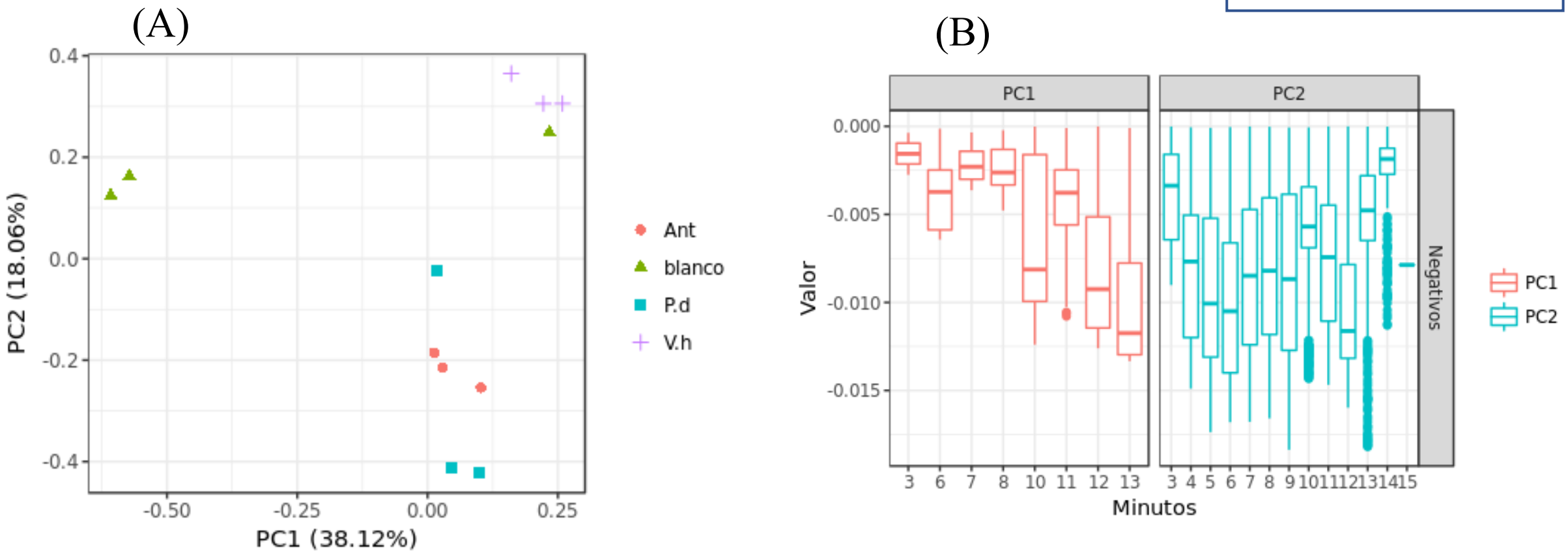

Figure. (A) Score plot of Sample type (B) loadings per minute

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Biomolecules of interest

Results & Discussion

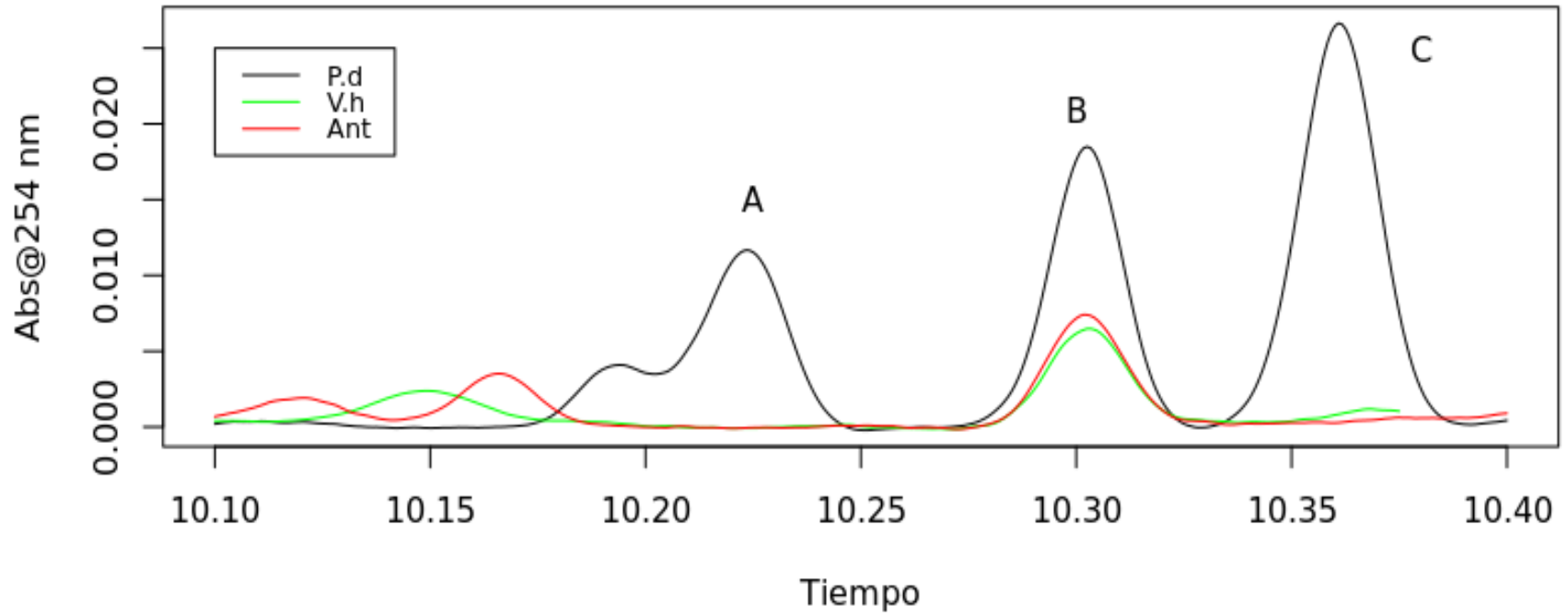

Figure. Identified biomolecules with possible implications in antagonism interaction.

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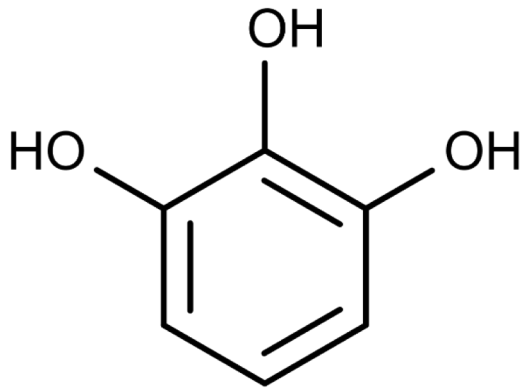
Organic Structure

Results & Discussion

254 nm

$\pi \longrightarrow \pi^*$

In conjugated systems



Pyrogallol

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Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 1567–1572
Bioorganic & Medicinal Chemistry Letters

Pyrogallol and its analogs can antagonize bacterial quorum sensing in *Vibrio harveyi*

Nanting Ni,[a] Gaurav Choudhary,[b] Minyong Li[a] and Binghe Wang[a,*]

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[b]*Department of Biology, Georgia State University, Atlanta, GA 30303, USA*

Received 24 December 2007; accepted 22 January 2008
Available online 30 January 2008

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Organic Structure

Results & Discussion

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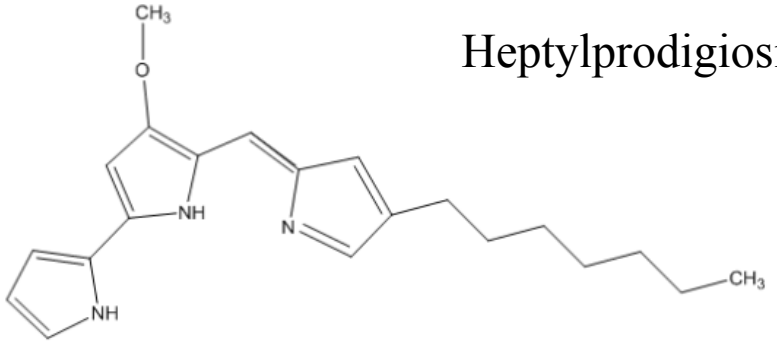
Heptylprodigiosin



Review

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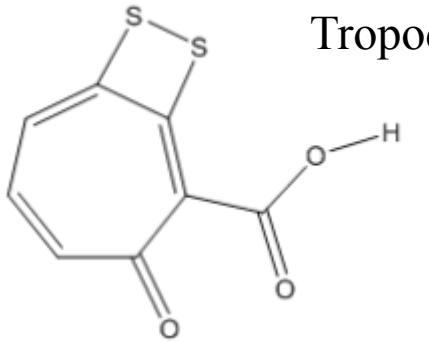
Tropodithietic acid



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[5] Crowley, S., O'Gara, F., O'Sullivan, O., Cotter, P., & Dobson, A. (2014). Marine Pseudovibrio sp. as a Novel Source of Antimicrobials. *Marine Drugs*, *12*(12), 5916-5929. doi:10.3390/md12125916

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Conclusions

Conclusions

- This study show a successful implementation of liquid chromatography to obtain untargeted metabolomic profiles of microbial antagonism interaction.
- Chemometrics algorithms have been employed correctly for signal handle, multivariate analysis and biomarkers discovery.
- Through supervised analysis, possible bioactive biological compounds have been identified.

Further work

- Isolation of identified biomolecules and conduction of biological essays egains *V. harveyi*.
- Structural elucidation by advanced analytical techniques such as IR, MS, NMR

Acknowledgement

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