Characterization of culturable marine luminescent bacteria isolated from the Gulf of Nicoya, Costa Rica

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Introduction

Luminescence in bacteria is dependent on quorum sensing and is regulated by the luxCDAB operon. The expression of this operon genes can be inhibited by exposition to toxic substances such as heavy metals.

The ratio between total heterotrophic culturable bacteria (THB) and luminescent bacterial fraction (FL) had been used as biosensor of coastal water quality (Bagordo et al. 2012).

Methodology

Surface water samples were taken at 1.0 m of depth from four sites of Gulf of Nicoya (Tropical Eastern Pacific)(Fig 1) during the 2015-2016 dry, rainy and two transitions seasons.

Total culturable heterotrophic (THB) and luminescent bacteria fraction (FL) were determined and the luminescent strains were identified by biochemical tests and multilocus sequence analysis (MLSA) using 16S rRNA, gyrB and pyrH genes (Cano-Gómez et al. 2011).

The effect of heavy metals on growth and luminescence was determined by disk diffusion test at different concentrations of cadmium, copper, lead and zinc.

Objectives

- Establish the abundance and species composition of luminescent bacteria.
- To evaluated the preliminary effect of heavy metals on luminescence intensity.

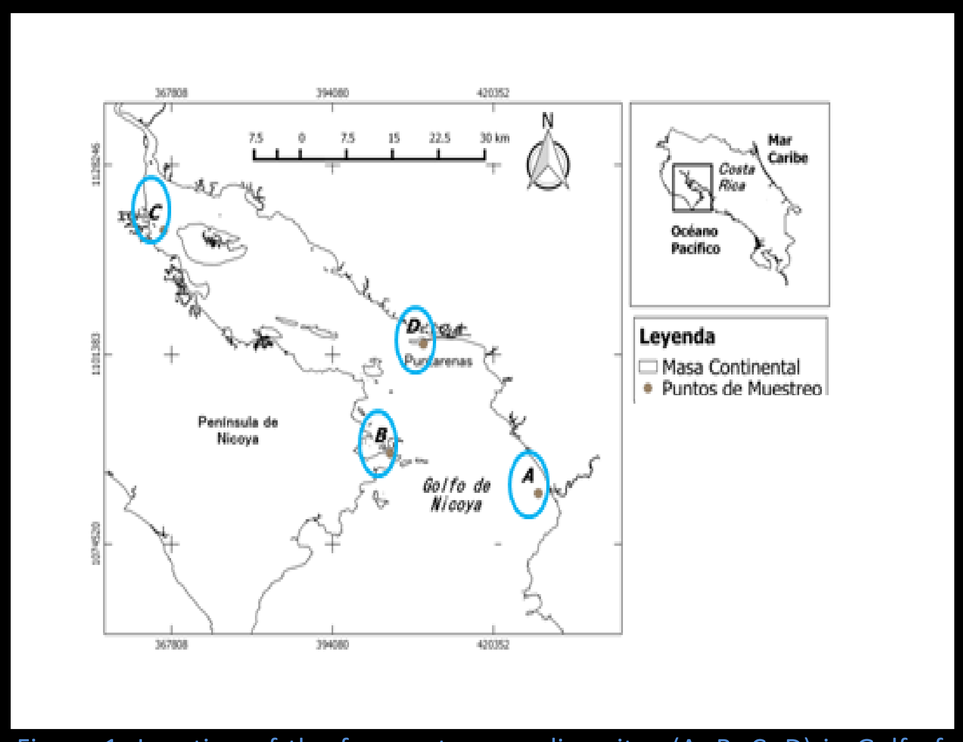


Figure 1. Location of the four water sampling sites (A, B, C, D) in Gulf of Nicoya, Costa Rica.

Results

- LF was ranged from 0-10% of total heterotrophic bacteria (Fig 2). No significant differences were registered among sites and seasons.

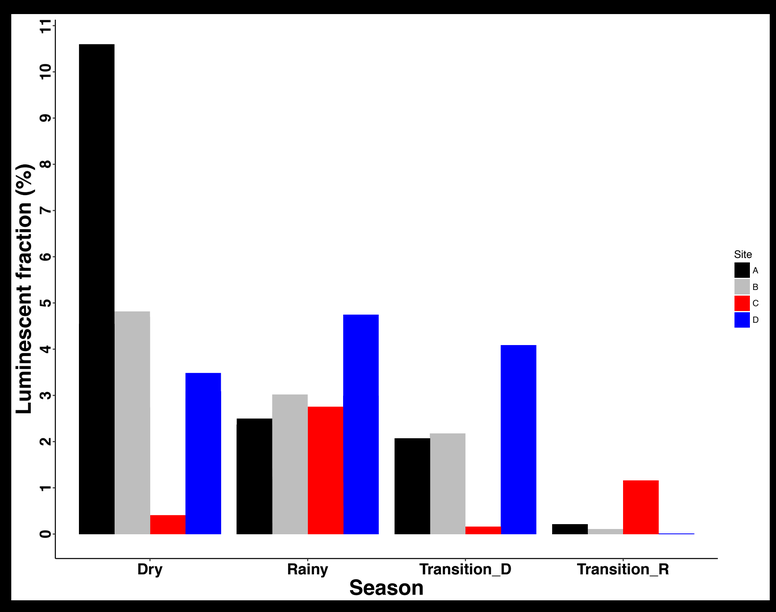


Figure 2. Abundance of luminescent bacteria expressed as a percentaje of the total heterotrophic bacteria (% LF).

Seq Match Algorithm (rRNA 16S)

Sample	Identification	Similarity (s_ab %)	GenBank accesion number
CL3	*Vibrio harveyi*	0.93	*EF635306*
CL6	*Vibrio rotiferianus*	0.89	*AJ316187*
CL8	*Vibrio harveyi*	0.94	*DQ146937*
CL11	*Vibrio harveyi*	0.99	*DQ146935*
CL18	*Vibrio harveyi*	0.92	*EU404191*
CL21	*Photobacterium leiognathi*	0.92	*FJ240415*
CL22	*Vibrio azureus*	0.94	*AB682212*
CL24	*Photobacterium leiognathi*	0.99	*AB243239*

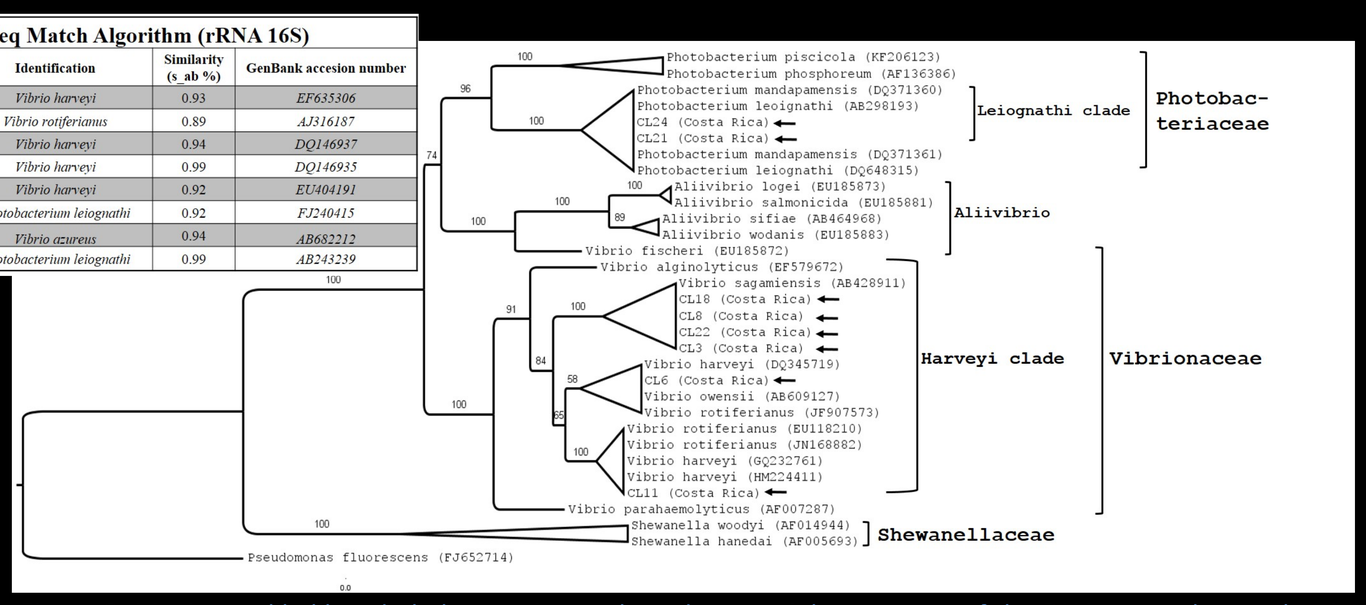
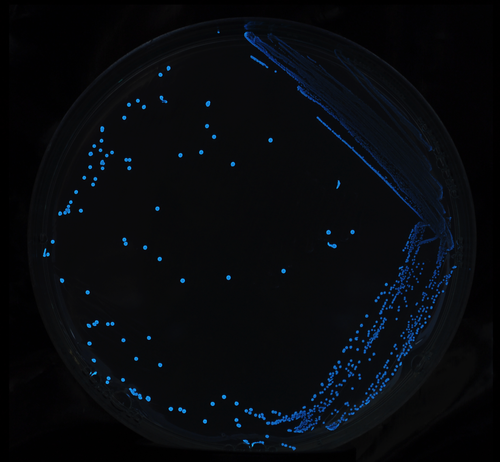


Figure 3. Maximum-likelihood phylogenetic tree based on partial sequences of the DNA gyrase beta subunit (gyrB) and sequence used in this assay (strains CL3, CL6, CL8, CL11, CL18, CL21 and CL24 with bold arrow). Numbers at the branches indicate bootstrap support (percentages of 1000 permutations)

- The luminescent strains were grouped in the Vibrionaceae and Photobacteriaceae familly (Fig 3).



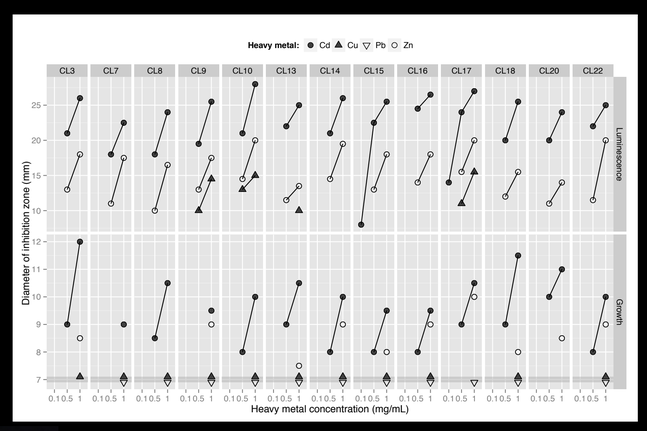


Figure 4. Heavy metal inhibition zone diameter (cm) on bacterial growth and luminescence.

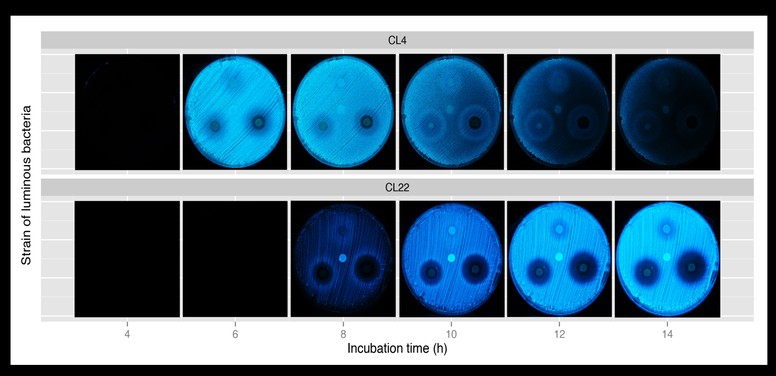


Figure 5. Effect of cadmiun at 0.1 (upper disc position), 0.5 (left disc position), 1.0 (right disc position) and 0.0 (central disc position) mg/mL on luminescence of two strains (CL4 and CL22).

- Higher inhibitory effect of heavy metals was observed on luminescence than on bacterial growth (Fig 4 and 5).
- Some of these strains have potential as biosensors, but further studies are needed

Refrences

Bagordo, F., Serio, F., Lugoli, L., Grassi, T., Idolo, A., Gabutti, G. & Donno, A. (2012). Phenotypic characterization of culturable marine luminous bacteria isolated from coastal waters of the southern Adriatic Sea (Otranto, Italy). *Ciencias Marinas*, 38*(4)*, 599–608.

Cano-Gómez, A., Høj, L., Owens, L. & Andreakis, N. (2011). Multilocus sequence analysis provides basis for fast and reliable identification of Vibrio harveyi-related species and reveals previous misidentification of important marine pathogens. Syst Appl Microbiol 34, 561-565.

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