

SHORT COMMUNICATION

## *In silico* analysis of *PirA*- and *PirB*-like toxin genes of *Vibrio* spp., present in Asia and Costa Rica

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### ABSTRACT

**Objective:** Acute hepatopancreatic necrosis disease is an emerging infectious disease of *Penaeus* species. The causative agent is *Vibrio* species, which dispels binary toxin similar to *pirA* and *pirB*, which causes mortality in infected shrimp. The aim of this research was to investigate the evolutionary relationship of *pirA* and *pirB* homologous genes present in this Asia and Costa Rica *in silico*.

**Materials and methods:** The sequences for *in silico* analysis were all retrieved from the Basic Local Alignment Search Tool Nucleotide (BLASTN) tool of the National Center for Biotechnology Center. For *pirA*, a total of 25 sequences submitted from different Asian countries and Costa Rica were retrieved for analysis. Meanwhile, for *pirB*, a total of 11 sequences submitted from five Asian countries were retrieved. Sequences were aligned using the CLUSTAL W alignment tool under Molecular Evolutionary Genetics Analysis (MEGA) 7 software. The evolutionary history was then estimated using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) method, whereas the evolutionary distances were determined using the maximum composite likelihood model with 1,000 bootstrap replications.

**Results and Discussion:** The results show that, among 27 DNA sequences analyzed for *pirA* gene, three groups were generated, while for *pirB*, 13 DNA sequences yielded only one group. The analysis revealed low genetic variation among isolates for both *pirA* and *pirB* genes.

**Conclusion:** This result suggests that the low frequency of polymorphism and geographic location cannot be attributed to the differences in *V. parahaemolyticus* isolates in Asian countries relative to Costa Rican isolates in *pirA* and *pirB* genes.

### ARTICLE HISTORY

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### Introduction

The acute hepatopancreatic necrosis disease (AHPND, formerly known as early mortality syndrome) has reportedly been the cause of high mortalities and production in Southeast Asian countries. It induces necrosis in the hepatopancreas of the diseased shrimp, which is caused by the bacterium *Vibrio* species [1].

Histological sections of the hepatopancreas of *Penaeus vannamei* (Fig. 1A and B) show the differences between the hepatopancreas of normal and infected shrimps. Clearly, AHPND lesions are present (Fig. 1B) through the immense sloughing of hepatopancreatic tubule epithelial cells into the lumen, which only suggests severe necrosis marked

by cellular detachment relating to a bacterial infection. Moreover, the distinct formation of melanized hemocytic nodules in the middle part of the hepatopancreas tubules is evident [1].

Recently, the reports on the pathogenicity of strains of *V. parahaemolyticus* indicate that there is only a specific region in the plasmid of the bacteria that determines the pathogenicity of the bacteria [2]. Several studies illustrate that the genomes of these pathogenic isolates possess genes, which are homologous with the Photorhabdus insect-related (Pir) toxin genes *pirA* and *pirB* [3].

The mechanism of speciation in bacteria remains to be a topic of interest among researchers worldwide. Aside from

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its unpredictability and not to mention huge data sets to be fully explored, single-point mutations can play a tremendous role in determining the evolutionary relationship of *Vibrio* species across different locations. A representative of such data sets on *pirA* and *pirB* genes can give a glimpse on the genomic data of these genes. Furthermore, since *in silico* analyses on *pirA* and *pirB* genes from Southeast Asia and Costa Rica have not yet been thoroughly studied, this study can examine the possible diversification and/or classification of *Vibrio* species.

In this paper, the *in silico* analysis of *pirA* and *pirB* sequences from Southeast Asia and Costa Rica available in the National Center for Biotechnology Information (NCBI) was done. The phylogenetic trees of *Vibrio* spp. sequences of *pirA* and *pirB* toxin genes were also constructed to determine the evolutionary relationship of each isolate in different countries.

## Materials and Methods

### DNA sequences used in this study

The sequences for *in silico* analysis were all retrieved from the Basic Local Alignment Search Tool Nucleotide (BLASTN) tool of the NCBI. All of the sequences that were used are the only available sequences in NCBI as of June 25, 2019. For *pirA*, a total of 25 sequences submitted from different Asian countries and Costa Rica were retrieved for analysis. Two *pirA* sequences from *Photobacterium luminescens* served as outgroup. For *pirB*, a total of 11 sequences submitted from five Asian countries were retrieved. Each sequence is labeled according to their corresponding accession numbers, followed by their country of origin.

### Sequence and phylogenetic analyses of *Vibrio* spp.

Sequences were aligned using the CLUSTALW alignment tool under Molecular Evolutionary Genetics Analysis (MEGA) 7 software. Then, the evolutionary history was inferred using Unweighted Pair Group Method with Arithmetic mean (UPGMA) method, whereas the evolutionary distances were

computed using the maximum composite likelihood model with 1,000 bootstrap replications. The analysis was conducted for 27 nucleotide DNA sequences for *pirA*, whereas 13 nucleotide sequences were used for *pirB*, which include two outgroups for each target gene.

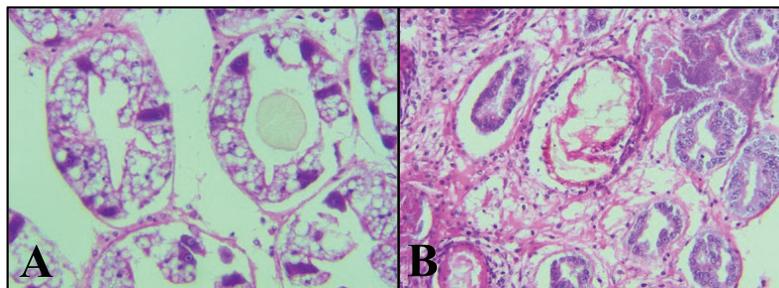
## Results and Discussion

### Sequence and phylogenetic analyses

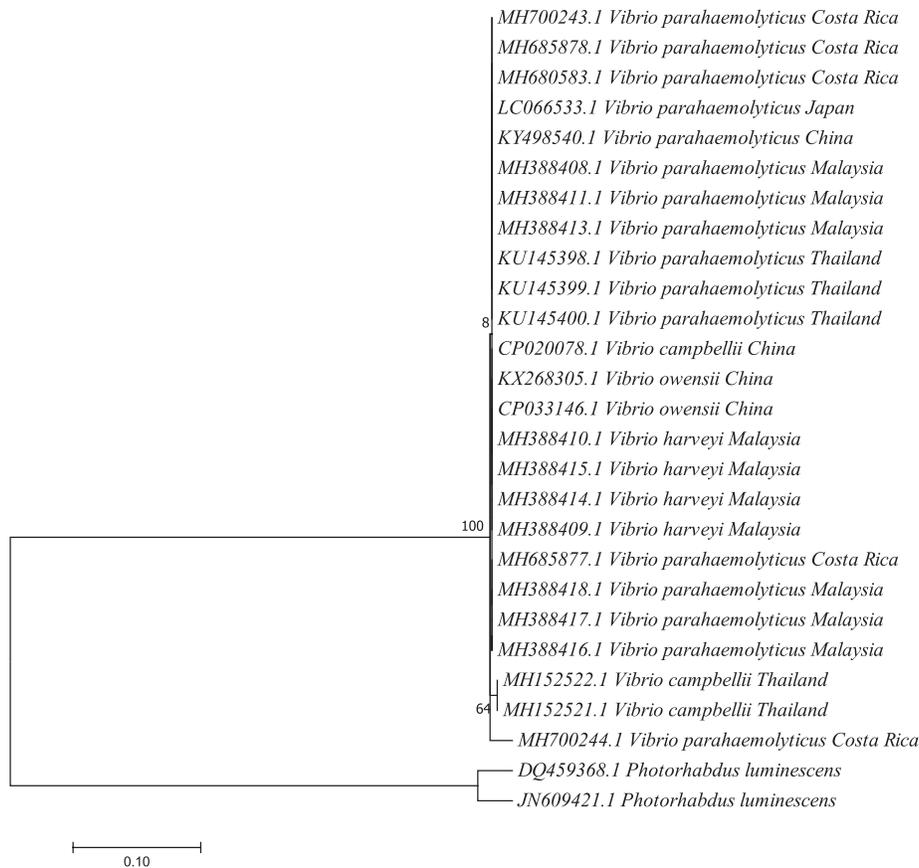
The *pirA* sequences of *Vibrio* spp. used in this study had an average of 306 nucleotide bases. Asian isolates range from 209 to 336 bp, whereas Costa Rican isolates range from 283 to 287 bp. The average nucleotide frequencies of Asian isolates (T = 25.10%, C = 21.20%, A = 30%, and G = 23.70%) differ from Costa Rican isolates (T = 24.60%, C = 21.46%, A = 28.78%, and G = 25.10%). The aligned sequences from the 25 *pirA* yielded 205 identical pairs, 32 transitional pairs, and 38 transversional pairs. On the other hand, *pirB* sequences had an average of 1,308 nucleotide bases (including the outgroup). The average nucleotide frequencies were as follows: T = 31.4%, C = 15.8%, A = 31.7%, and G = 21.1%. A low Guanine and Cytosine (GC) content of both *pirA/B* indicates recent acquisitions of these genes [4]. The high similarity between sequences was observed, having 1,010 base similarity, 103 transitional pairs, and 123 transversional pairs. This only suggests that, for *pirB*, low variation among isolates was observed due to high similarity.

As shown in Figure 2, phylogenetic analysis revealed the sequences of *pirA* genes from different *Vibrio* spp. from the isolates of Asian countries and Costa Rican. The *pirA* sequences from different *Vibrio* spp. include *V. harveyi*, *Vibrio owensii*, *V. campbellii*, and *V. parahaemolyticus* as shown in Figure 2.

This figure showed a distinct tree topology for *pirA*, which attained high nodal bootstrap support. It clearly illustrates a well-known property of bootstrapping: high nodal bootstrap of 100 was generated in the main root, hence indicating that the optimal tree would be unlikely to change as sequence length increases. The constructed tree generated



**Figure 1.** Hematoxylin and H&E stained histological cross-sections of the hepatopancreas of *P. vannamei* sample. (A) Normal shrimp hepatopancreas tubule. (B) AHPND pathology. Actual photos were taken from the Bureau of Fisheries and Aquatic Resources, National Fisheries Laboratory Division.



**Figure 2.** Phylogenetic tree of *Vibrio* spp. isolates targeting *pirA* gene from Malaysia, China, Japan, Thailand (Asia), and Costa Rica.

three distinct groups and one outgroup. Interestingly, Asian and Costa Rican isolates were grouped together for *pirA* gene despite differences in geographic location, thus corroborating with the previous studies that toxin genes such as *pirA* and *pirB* exhibit low polymorphism. This result only suggests that the low frequency of polymorphism and geographic location cannot be attributed to the differences in *V. parahaemolyticus* isolates in Asian countries relative to Costa Rican isolates in *pirA* gene. It answers the query of Peña-Navarro et al. [5] about the similarity of Costa Rican isolates than that of Asian genotypes. The phylogenetic relationship between these isolates has not been reported yet and thoroughly studied. The isolates of *V. campbellii* and *V. owensii* from Vietnam and China, respectively, were shown to cause AHPND in 2015 [6,7]. These isolates were confirmed to comprise a plasmid that is analogous to the one discovered in AHPND-causing *V. parahaemolyticus* which produces the binary toxins similar to *pirA/pirB* [8–10].

Consequently, it supports the theory that *pirA/pirB* are the main virulence factors that cause AHPND.

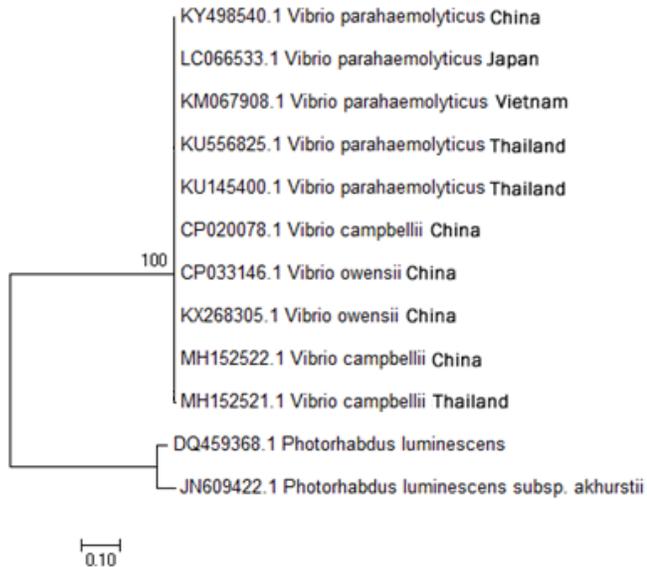
It clearly shows that horizontal gene transference (HGT) occurs between sister species belonging to closely related clades, as argued by Sawabe et al. [11] in their previous

study. The results generated from this study will impact the shrimp industry in terms of analyzing *pirA/pirB* toxin genes present in isolates with AHPND.

A phylogenetic analysis of *pirB* showed that Asian isolates have a high similarity to each other. Clearly, based on *pirB* sequences, *Vibrio* species in Asia are grouped together with 100% bootstrap value. A sample size of *pirB* is smaller compared to *pirA* since few sequences are deposited in NCBI, whereas no sequences from Costa Rican isolates were retrieved in this study. The phylogenetic tree formed a monophyletic group and one outgroup.

As shown in Figure 3, the genetic variation is low among geographic isolates of *Vibrio* spp. and consistent with Figure 2 from *pirA* phylogenetic tree. Clearly, within the species level, *Vibrio* species are grouped together in one clade. This analysis could have better findings if there are more *pirB* sequences retrieved from NCBI.

Following the genetic variations of sequences from *pirA* and *pirB*, these will help in interpreting the evolution of this AHPND-causing *Vibrio* spp., which is essential for diagnostic and management strategies up to date, and subsequently, to reduce the spread of the disease and lessen its impact on commercial shrimp farms.



**Figure 3.** Phylogenetic tree of *Vibrio* spp. isolates targeting *pirB* gene from China, Japan, Vietnam, and Thailand.

## Conclusion

DNA sequences were analyzed *in silico* for *pirA* and *pirB* of *Vibrio* species in Asia and Costa Rica. The results showed low genetic variability among all the geographic isolates of *Vibrio* spp. for the genes such as *pirA* and *pirB*. This result only suggests that the low frequency of polymorphism and geographic location cannot be attributed to the differences in *V. parahaemolyticus* isolates in Asian countries relative to Costa Rican isolates in *pirA* and *pirB* genes. Furthermore, it can be good evidence that HGT is the mode of transmission, in which *pirA/B* can be spread interspecifically. An increase in sample size for *pirB* gene is highly recommended for further study to give comprehensive information on the phylogenetic relationship of *Vibrio* species across different countries.

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## Conflict of interest

The authors declare no conflict of interest.

## Authors' contributions

The first author and the corresponding author both conceptualized and conducted this study. The second author is responsible for the analyses in this study.

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