LETTER TO THE EDITOR

An isolate of *Vibrio campbellii* carrying the *pir*<sup>VP</sup> gene causes acute hepatopancreatic necrosis disease

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Dear Editor,

In recent years, acute hepatopancreatic necrosis disease (AHPND) has rapidly spread in Asian countries and Mexico, causing severe mortality (up to 100%) and decreasing shrimp production. AHPND was originally shown to be caused by a specific virulent strain of *Vibrio parahaemolyticus*, namely the AHPND-causing *V. parahaemolyticus* (VPAHPND)<sup>1,5,6</sup>. *V. parahaemolyticus* becomes virulent VPAHPND after acquiring a plasmid (pVA1) expressing the deadly toxin *pir*<sup>VP</sup>, which consists of two subunits, PirA and PirB, and is homologous to the Pir (Photorhabdus insect-related) binary toxin.<sup>7</sup> The plasmid pVA1 also carries a cluster of genes related to conjugative transfer; hence, this plasmid may potentially be able to transfer not only among *V. parahaemolyticus* strains but also to different bacterial species.<sup>7,10</sup> So far, there have been no published reports directly demonstrating that *Vibrio campbellii* can harbor *pir*<sup>VP</sup> and cause AHPND in shrimp. In this paper, we challenged *Litopenaeus vannamei* with a strain of *V. campbellii* (20130629003S01) carrying *pir*<sup>VP</sup> isolated from a *L. vannamei* farm and demonstrated that *V. campbellii* is a causative agent of AHPND.

In this paper, strain 20130629003S01 was isolated in June of 2013 from diseased *L. vannamei* in Guangxi, China. PCR and RT-PCR amplifications were performed using VpPirA and VpPirB primers specific to *pir*<sup>VP</sup> genes (*pirA* and *pirB*).<sup>11</sup> The electrophoresis of PCR products showed that both *pirA* (284 bp) and *pirB* (392 bp) were detected in the strain (Figure 1A). A partial sequence of 16S rRNA was obtained by sequencing the PCR products obtained with primers 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492R (5′-TAC GGC TAC CTT GTT ACG ACT T-3′),<sup>12</sup> and the pathogenicity of strain 20130629003S01 was examined in healthy *L. vannamei* shrimps weighing ~1 g, which were reared in 90 l artificial seawater at salinity 30 in plastic tanks (density 15 shrimps/tank) at 27 ± 2°C. An immersion challenge was used to follow the bioassay protocol described by Tran et al.<sup>5</sup> All experimental groups were assayed in triplicate. Shrimp immersed with the bacterial suspension began to develop typical gross signs of AHPND within 12 h, massive mortalities occurred from 12 h post challenge, and cumulative mortalities reached 100% within 36 h. Gross signs of challenged *L. vannamei* included an empty stomach and gastrointestinal tract as well as pale and atrophied hepatopancreas (Figure 1D). A histopathological examination of moribund shrimp revealed the presence of AHPND lesions (Figure 1E) characterized by the acute sloughing of hepatopancreatic tubule epithelial cells, some of which displayed intact organelles, such as nuclei and cytoplasmic vesicles (Figure 1E). To our knowledge, our study is the first to demonstrate that a *V. campbellii* strain carrying *pir*<sup>VP</sup> causes AHPND. Therefore, AHPND caused by non-*V. parahaemolyticus* should be further investigated.

The shrimp farming industry is one of the important economic industries for countries in Asia and Latin America. AHPND is characterized by the acute and massive mortality in shrimp farms,

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Effective biosecurity measures should be considered to prevent the spread of V. parahaemolyticus isolates has been reported in a Vibrio harveyi-like strain from Vietnam and a Vibrio owensii-like strain from China. The present results may provide evidence for the horizontal transfer of the pir\textsuperscript{VP} gene or PVAl plasmid between different bacterial species, thereby potentially increasing the complexity of causative agents of AHPND and aggravating the threat to the shrimp industry. On the basis of our finding that a V. campbellii carrying pir\textsuperscript{VP} causes AHPND, effective biosecurity measures should be considered to prevent the spread of AHPND in the future.

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