Role of PirB^{vp} lectin and PirA^{vp} toxin of *Vibrio parahaemolyticus* to promote the Acute Hepatopancreatic Necrosis Disease (AHPND) in shrimp via glycosylation-dependent PirB^{vp}-receptor interactions

Victorio-De Los Santos Marcelo[1]; Vibanco-Pérez Norberto[2]; Durán-Avelar Ma de Jesús[2]; Zenteno Edgar[3]; Soto-Rodríguez Sonia[1]

[1)]Food and Development Research Center-Mazatlán Unit. Av. Sábalo Cerritos S/N A.P. 711. Mazatlán, Sinaloa, México. marcelo.victorio@estudiantes.ciad.mx

2)

[3)] Department of Biochemistry of Medicine Faculty, National Autonomous University of Mexico.

ABSTRACT

Here, we show that lectin PirB^{vp} of Vp, a new lectin identified in this bacterium, is constitutively secreted together with PirA^{vp}, binding to the receptor to promote the AHPND. Through glycosylation-dependent mechanisms involving recognition of bacterial glycoproteins and glycosylated host cell receptors, PirB^{vp} enhanced Vp attachment to HP epithelial cells. Exposure to PirB^{vp}/PirA^{vp}, mainly in its tetrameric form, facilitated bacterial infection and theoretically produced the sloughing of tubule epithelial cells of the shrimp HP. This is the first evidence that show that the lectin PirB^{vp} of Vp binds to galactosamine/glucosamine receptor, interactions that can modulate the infection.

INTRODUCTION

A specific strain of *Vibrio parahaemolyticus* (Vp) is the etiological agent of an emerging shrimp disease, the acute hepatopancreatic necrosis disease (AHPND). Since 2009 to date, *Penaeus vannamei* shrimp production has suffered significant losses in Asian countries and Latin America including México, due to this disease. The mechanism of infection of Vp to recognize the receptor or adhesion molecule in the membrane of hepatopancreas (HP) to cause the death of shrimp, is unknown. However, Vp has evolved multiple strategies to promote adhesion and/or colonization of host cells, including those involving both bacterial and host glycans.

METHODOLOGY

The dialyzed 80% ammonium sulfate precipitates from broth cultures of 17 hours of isolates of Vp AHPND^{+} were also subjected to SDSD-PAGE to identified PirA^{vp} and PirB^{vp} toxins. The proteins recombinants were cloned into the plasmid pET28a and pET32c respectively, transformed with *E. coli* BL21 RIIL, induced with 0.5 mM of IPTG and purified with a column Ni-NTA Agarose. The SDS-PAGE were stained with silver nitrate or blotted onto nitrocellulose membranes for immunodetection with specific antibodies generated in rabbits by our laboratory. Using fluorescence based thermal shift assays, we studied the effects of pH and additives ions on the stability of PirA^{vp} and PirB^{vp}. The hemagglutinating activity of PirA^{vp} and PirB^{vp} toxins was tested by using rat red blood cells.

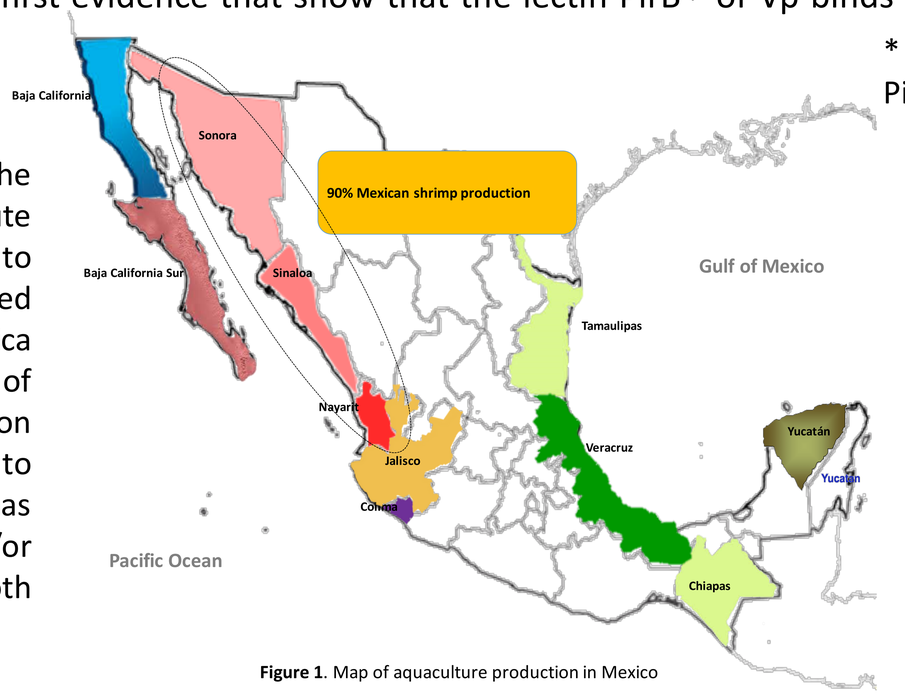


Figure 1. Map of aquaculture production in Mexico

RESULTS

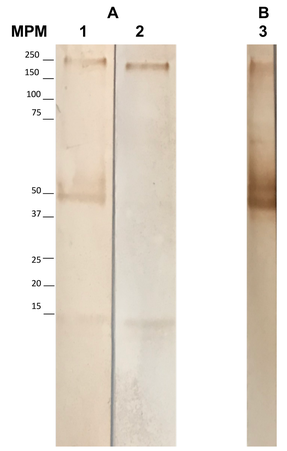
* Interaction of cell membrane proteins of rat red blood with $_{r}$PirB^{vp} and their possible synergy molecular with $_{r}$PirA^{vp}

Table 2. Hemmagglutinating activity assay of PirA^{vp} and PirB^{vp} toxins

	Specific activity		
Red cells	$_{r}$PirA^{vp}	$_{r}$PirB^{vp}	PiA^{vp}/PirB^{vp} complex
Humman A	NA	NA	NA
Humman B	NA	NA	NA
Humman 0	NA	NA	NA
Rabbiit	NA	NA	NA
Mouse	NA	NA	NA
Rat	NA	81,476	127,600
Ovine	NA	NA	NA

PirA^{vp} concentration was 1.12 μg/25 μL, PirB^{vp} 0.8 μg/25 μL and PirA^{vp}/PirB^{vp} complex 0.5 μg/25 μL. (NA) No Hemmagglutinating activity. Hemagglutinating activity is reported as the inverse of the last dilution showing visible agglutinating activity. This assays were also performed in the presence of 2 % erythrocytes suspension in PBS pH 7.4.

* Polyclonal Antibodies recognized oligomeric forms of PirA^{vp} and PirB^{vp}.



* Oligomerization structures of PirA^{vp} and PirB^{vp} toxins

CONCLUSIONS

It is necessary presence and oligomerization of pirA^{vp} to recognize the receptor. Pir B^{vp} binds to the receptor most likely forming a complex with PirA^{vp} and oligomerizing as a tetramer.

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