Int J Pharm Bio Sci 2013 Jan; 4(1): (B) 592 - 595

Research Article **immuno biology**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

EXPRESSION OF TOLL PROTEIN GENE IN *LITOPENAEUS VANNAMEI* (PACIFIC WHITE SHRIMP) EXPERIMENTALLY INDUCED WITH *VIBRIO ALGINOLYTICUS*

SOWMYA. M.V, UMA. A* AND REBECCA. G

Shrimp Disease Diagnosis Laboratory, Vaccine Research Centre – Viral Vaccines, Centre for Animal Health Studies, Tamilnadu Veterinary and Animal Sciences University, Madhavaram Milk colony, Chennai – 600051, India

ABSTRACT

Toll proteins are components of innate immune system known to be involved in the recognition of pathogen associated molecular patterns (PAMPs), the conserved motifs of the pathogens and in eliciting the immune response. This study was carried out with an objective to identify the expression of Toll protein gene in *Litopenaeus vannamei* and to compare its expression profiles in healthy and experimentally induced status. *L.vannamei* was experimentally induced by exposure to *Vibrio alginolyticus* at a concentration of 9×10^{12} colony forming units/ml (CFU ml^{-1}). Tissues of various organs *viz*., muscle, gut, gills, heart, nerve and pleopods of *L.vannamei* that were healthy and experimentally exposed with *V.alginolyticus* were used to study the toll protein gene expression profile by Reverse transcriptase Polymerase chain reaction (RT- PCR). Basal expression of toll protein gene at various levels was observed in muscle, gut, gills, heart, nerve and pleopods of healthy *L. vannamei*. Comparatively higher levels of toll protein gene expression were observed in the tissues of *L. vannamei* that were experimentally exposed with *V.alginolyticus,* the highest induced expression level was recorded in the nerve tissue.

KEY WORDS: ***Litopenaeus vannamei*, toll protein, expression, experimental induction, *Vibrio alginolyticus*.**

UMA. A
Shrimp Disease Diagnosis Laboratory, Vaccine Research Centre – Viral Vaccines, Centre for Animal Health Studies, Tamilnadu Veterinary and Animal Sciences University, Madhavaram Milk colony, Chennai – 600051, India

INTRODUCTION

Toll proteins are a class of proteins that play a vital role in the innate immune system[1] as they are involved in the recognition of conserved motifs in pathogens called Pathogen Associated Molecular Patterns (PAMPs). Shrimp are marine invertebrates which largely depend on the innate immune system to protect themselves against the invading pathogens. *L.vannamei* (Pacific white shrimp) is the widely farmed shrimp species globally. Disease outbreaks are the major constraint to the operation of shrimp farms and hatcheries. Vibriosis is a bacterial disease responsible for mortality of cultured shrimp worldwide[2 3 4]. *V.alginolyticus* have been reported to cause septicemia [5,6] and shell diseases[7] in marine shrimp. As toll proteins recognize the pathogens and elicit the immune protection mechanism against infections, this study was carried out with an objective to identify the expression of toll protein gene and to compare the expression profiles in various organs of *L.vannamei* in healthy and experimentally induced (*V.alginolyticus)* status.

MATERIALS AND METHODS

(1) L.vannamei samples

L.vannamei juveniles were procured from a commercial shrimp farm in Nellore, Andhra Pradesh. The shrimps were maintained in an aerated tank at 22-25ºC with adequate feeding. A confirmed isolate of *V.alginolyticus* isolated (VAC/SDDL/11) from *Penaeus monodon* showing clinical symptoms of bacterial shell disease collected from a shrimp farm in Minjur, Tamilnadu, India was used. The isolate was inoculated in Tryptic soya broth (with 1% saline) and grown overnight at 30ºC. The bacterial culture was centrifuged, enumerated and used for immersion exposure to *L.vannamei*[8]. Formalin inactivation of bacterial culture was carried out and used for the experiment[9].

(2) Experimental exposure of L.vannamei with V. alginolyticus

L.vannamei were experimentally exposed to *V. alginolyticus* at a concentration of 9×10^{12} colony forming units (CFU $ml^{-1)}$ of the rearing medium. After one hour exposure, the animals were sacrificed and the organs such as gills, stomach, hepatopancreas, gut, muscle and heart were collected and used immediately for total RNA extraction. Samples were also collected as above from the control group maintained without experimental exposure. Total RNA was extracted from the tissue samples using a commercial RNA extraction kit (BioBasic INC., Canada). RNA from samples was reverse transcribed to cDNA separately using high capacity cDNA synthesis kit (Applied Biosystems Inc, USA).

(3) PCR amplification of Toll protein gene

About 1µl (6 µg) of cDNA was used as the template for PCR amplification of toll protein gene, using self-designed PCR primers SDDL/TL/PV380F and SDDL/TL/PV380R. The primers were designed based on the sequence information of Toll protein gene of *L.vannamei* available in GenBank (Acc.no.DQ923424). PCR assay was carried out in a total volume of 25µl with 22µl of PCR master mix (Bangalore Genei Pvt Ltd), 1µl each of forward and reverse primers (30pmoles) and 1µl of cDNA. PCR amplification was carried out with an initial denaturation for 5min at 94ºC followed by 30 cycles of 1 min at 94ºC, 55ºC for 1min, 72ºC for 1 min with a final extension for 7min at 72ºC. The PCR products were analyzed by standard gel electrophoresis with 100bp DNA marker using 2% agarose gel stained with ethidium bromide, visualized and documented in a gel documentation unit (Vilber-Lourmet, France).

RESULTS

The results of this study showed that the basal

expression of toll protein gene expression is present in healthy shrimp and it is induced when experimentally exposed with *V. alginolyticus.* PCR amplification of toll protein gene from tissues of healthy *L.vannamei* from control group resulted in the expected product size of 380bp size indicating the presence of basal expression at various levels in muscle, gut, gills, heart, nerve and pleopods. The normal or basal expression of toll-like receptors or toll proteins in tissue implicates its ability to resist the pathogen challenge[10]. The toll protein gene expression levels in experimentally induced group were comparatively higher than the control group as evidenced from the visual observation of the intensity of PCR amplified products (Fig . I). The levels of expression of toll protein gene varied in the tissues with the highest in the nerve tissue followed by pleopods, gills, heart and muscle.

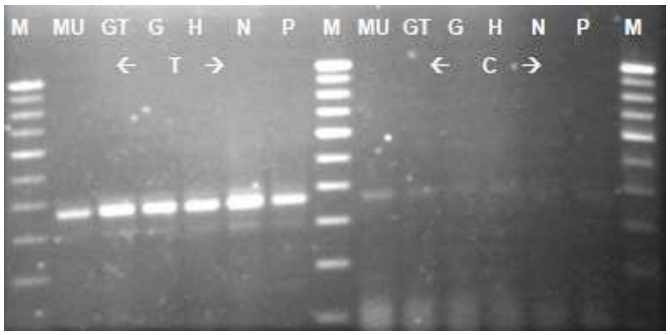

Figure 1
PCR amplified products of Toll protein gene of L. vannamei
C - Healthy control L. vannamei; T – L. vannamei experimentally induced with V.alginolyticus
Lanes: M - 100bp molecular weight marker; MU – Muscle, GT – Gut, G – Gills, H – Heart, N-Nerve and P-Pleopods.

DISCUSSION

Basal expression of toll protein gene have been reported in various organs *viz.,* gill, gut lymphoid organ, heart, hematopoietic organ, hemocytes, ventral abdominal nerve cord, eye stalk, neural ganglia and brain of *Marsupenaeus japonicus*[11]. Earlier studies have shown that the expression levels of toll proteins were not the same in all the tissues as the transcription levels of toll protein in *P.monodon* was higher in hepatopancreas and gut tissue than in gills[12]. *Fenneropenaeus chinensis* induced with *V.anguillarum* also showed higher expression of toll in lymphoid organs and gill which is attributed to the predominant participation of these organs in shrimp immune activities[8]. The expression levels of Toll protein gene varies with the tissues of the organs depending on its involvement in immunity as evident from the differential expression of Toll protein gene in tissues of *L.vannamei* induced with same concentration of *V.alginolyticus* in this study.

CONCLUSION

Inducing the immunity in *L.vannamei* by experimental exposure with bacterial ligands like inactivated *V.alginolyticus* will help in bringing about TLR based immuno protection against diseases in shrimp and to avoid production and economic losses in shrimp culture due to disease caused by *V.alginolyticus*.

ACKNOWLEDGEMENTS

The first author gratefully acknowledges the university officers of Tamil Nadu veterinary and animal science university, Chennai, India for extending the research facilities of shrimp disease diagnosis laboratory to carry out this research work as a part of M.Tech degree program.

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