Indian J. Fish., 62(3) : 151-156, 2015

Note

Messenger RNA expression of pattern recognition receptor proteins in the pearl oyster *Pinctada fucata* (Gould, 1850) in response to lipopolysaccharide stimulation

A. ANJU, J. JESWIN, P. C. THOMAS, M. P. PAULTON AND K. K.VIJAYAN
Marine Biotechnology Division, ICAR-Central Marine Fisheries Research Institute, P B. No. 1603 Ernakulam North P. O., Kochi - 682018, Kerala, India
e-mail: anju.moonjely@gmail.com

ABSTRACT

Pattern recognition receptor proteins (PRP) are capable of binding specifically to conserved portion of microbial cell wall components and they are involved in the recognition of different immune modulators. Proteins such as F-type lectin, galectin and LGBP play protective role in innate immunity of invertebrates. The time dependent expression of F-type lectin gene as well as galectin and LGBP genes was determined after lipopolysaccharide (LPS) challenge in the pearl oyster *Pinctada fucata*. A significant increase of mRNA expression levels of all the three genes under study was observed in haemocytes, reaching a maximum level at 8 h post-challenge and then declined to basal levels at 36 h. These results suggest that PRPs play a critical role in the innate immune system of the pearl oyster *P. fucata*.

Keywords: Lipopolysaccharide, mRNA expression, Pattern recognition receptor proteins, *Pinctada fucata,* Semi-quantitative PCR

Immune system protect multi-cellular organisms from foreign invaders There are two types of immunity in vertebrates, one providing innate (natural) immunity and the other providing adaptive (acquired) immunity. Pearl oysters, like other invertebrates lack adaptive immune system and exclusively rely on the innate immune response to combat invading organisms (Iwanaga and Lee, 2005). The innate immune system is designed to recognise molecules shared by groups of related microbes that are essential for the survival of those organisms and are not found associated with mammalian cells. These unique microbial molecules are called pathogen associated molecular patterns (PAMP) and include lipopolysaccharides (LPS) from the Gram negative cell wall, peptidoglycan (PG) and lipoteichoic acids from the Gram positive cell wall. In order to recognise PAMPs, various body cells have a variety of corresponding receptors called Pattern-recognition receptor proteins (PRPs) which are capable of binding specifically to conserved portions of these molecules (Raetz *et al*., 1991; Ulevitch and Tobias, 1995). Once an invading pathogen gain entry into the body of the host, they encounter a complex system of innate defense mechanisms involving cellular and humoral responses.

The present study focused mainly on three PRP genes *viz*., F-type lectin, galectin, LPS and β, 1,3-glucan binding protein (LGBP). Lectins are the best characterised PRPs and consist of a wide range of carbohydrate binding proteins of non-immune origin (Sharon and Lis, 2004; Vasta and Ahmed, 2008). Moreover, they present great structural diversity and a variety of carbohydrate affinity (Naganuma *et al*., 2006). Based on the primary structure, structural fold and cation requirement, animal lectins can be classified into several families, including C, F, P and I-type lectins, galectin, pentraxin and others (Honda, *et al*., 2000; Vasta, *et al.,* 2004). F-type lectins were first identified and characterised in European eel, *Anguilla anguilla* agglutinin (AAA) that has been used extensively as a reagent in blood typing and histochemistry. F-type lectins have been identified and described as immune recognition molecules in Japanese horseshoe crab *Tachypleus tridentatus* (Salto *et al*., 1997), Japanese eel *Anguilla japonica* (Honda *et al*., 2000), striped bass *Morone saxatilis* (Odom and Vasta, 2006), gilthead bream *Sparus aurata* (Cammarata *et al*., 2012), European seabass *Dicentrarchus labrax* (Salerno *et al*., 2009) and pearl oyster *Pinctada martensii* (Chen *et al*., 2011). In *Crassostrea* species, F-lectin is the main functional domain of binding for recognition during fertilisation (Moy and Vacquier, 2008).

Galectins constitute a relatively homogeneous lectin family and virtually all members bind β-galactosyl residues (Liao *et al*., 1994; Gauthier *et al*., 2002; Vasta *et al*., 2004). Galectins mostly recognise endogenous ligands and indirectly participate in inflammation and adaptive immunity by mediating chemotaxis, apoptosis

and developmental and regulatory aspects of adaptive immune responses (Gauthier *et al*., 2002; Hernandez and Baum, 2002; Rabinovich *et al*., 2002; Leffler *et al*., 2004; Chen *et al*., 2006; Hsu *et al*., 2006; Barrionuevo *et al*., 2007; Toscano *et al*., 2007). However, in invertebrates, our knowledge of the biological roles of galectins in innate immunity is very limited and fragmentary (Vasta *et al*., 2004). A multidomain galectin of eastern oyster *Crassostrea virginica* (CvGal) is responsible for recognising the protozoan parasite *Perkinsus marinus* (Tasumi and Vasta, 2007). Another multidomain galectin, AiGal from bay scallop *Argopecten irradians*, was also characterised and shown to be involved in innate immune responses (Song *et al*., 2011). Galectin has been identified and characterised in Pacific oyster *Crassostrea gigas* (Yamaura *et al*., 2008), Manila clam *Venerupis philippinarum* (Kim *et al*., 2008), abalone *Haliotis discus hannai* (EF392832), freshwater snail *Biomphalaria glabrata* (Yoshino *et al*., 2008) and pearl oyster *Pinctada fucata* (Zhang *et al*., 2011a,b).

Structural studies of LGBP have shown that the active site is occupied with Arg–Gly–Asp amino acids, which are responsible for the pattern recognition mechanism during adverse conditions. Basically LGBP is a glycosylated protein, which has the ability to bind with the glycosylated substrates like carbohydrate moieties. Studies on *Drosophila* demonstrated that LGBP functions as a recognition receptor for LPS and β-1, 3 glucan (Kim *et al*., 2000). Guanine nucleotide binding proteins (GNBP) otherwise known as LGBP, were originally purified from the silkworm *Bombyx mori* (Lee *et al*.,1996) and *Drosophila melanogaster* (Kim *et al*., 2000). LGBP has been cloned and characterised in pearl oyster, *Pinctada fucata* (Zhang *et al*., 2010); shrimp *Penaeus stylirostris* (Roux *et al*., 2002); scallop *Chlamys farreri* (Su *et al*., 2004) and shrimp *Litopenaeus vannamei* (Cheng *et al*., 2005). The aim of the present study was to determine changes in the mRNA expression levels of PRP genes *viz*., F-type lectin, galectin and LGBP by LPS stimulation in *Pinctada fucata*.

Live individuals of adult *P. fucata* (4.5-5.5 cm in shell length and 20-30 g body weight) were collected from a pearl farm in Thoothukudi, Tamil Nadu and maintained at 25˚C in tanks containing static aerated seawater (0.5 l per oyster) in the laboratory. Seawater was changed every day and the pearl oysters were fed with *Isochrysis galbana* twice daily. Animals were acclimatised for 2 weeks prior to initiation of experiment.

To study the time dependent expression of PRP genes, the experimental animals were exposed to LPS and the transcription levels were monitored by semi-quantitative PCR. The LPS stimulations were carried out in one group of animals (n=5) by injecting 50 µl of LPS (*Escherichia coli* 055:B5, #62326, Sigma–Aldrich, Munich, Germany) dissolved in PBS (LPS 10 µg ml^{-1}) into the adductor muscles of each pearl oyster. The control group (n=5) was injected with 50 µl of PBS alone. At each time point (0, 4, 8, 12, 24 and 36 h), haemolymph was collected using a syringe and immediately centrifuged at 5000 *g* at 4˚C for 10 min to harvest the haemocytes. Five individuals of each replicate were randomly sampled at the same time point. The haemocyte pellets from each individual were immediately used for RNA extraction.

From the haemocyte pellets, 200 ng of total RNAs were extracted using NucleoSpin RNA II reagent (MACHEREY-NAGEL GmbH & Co., Germany) and the extracts stored at -80°C until further use. cDNA was synthesised from RNA with iScript cDNA synthesis (Bio-rad). Semi-quantitative PCR was conducted with the cDNA to determine the relative expression of F-type lectin, galectin, LGBP and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA in haemocytes of pearl oyster. Primers for the PCR were designed from the full length sequence of F-type lectin (Anju *et al*., 2013) and from the published sequence of galectin (FJ812171), LGBP (FJ775601) and GAPDH (AB205404) genes of the *P. fucata* as shown in Table 1. The GAPDH was amplified in each PCR reaction as a loading control. The PCR products from different cycles of amplification were separated by gel electrophoresis in 1.5% agarose containing ethidium bromide (0.5 µg $µl^{-1}$) and visualised using a UV transilluminator. The cycle numbers that generate half-maximal amplification were used for subsequent quantitative analysis of gene expression *i.e.,* 30 cycles for F-type lectin, galectin and LGBP and 25 cycles for GAPDH.

Duncan's multiple comparisons test was used to compare significant differences in F-type lectin, galectin and LGBP gene expression between control and challenged samples using SPSS13.0 software. Differences were considered significant at $p<0.05$.

P. fucata is economically important for aquaculture along the south-east coast of India, and therefore, current problems associated with crop failure need to be resolved.

Table 1. Primers used in this study

Primer	Sequence (5`- 3`)
F-type lectin-F	TGGATGGTATAAGTAAT
F-type lectin-R	TCTGTTCGTTATTCTGAT
Galectin-F	AGATTTCCCCTTCAGTCCTTTC
Galectin-R	TGAAGAAATTGCATTCATGGAC
LGBP-F	CACACAGCAAGCCCCTGATCC
LGBP-R	CCTCCTCCGCCAGTTTGAGATG
GAPDH-F	TATTTCTGCACCGTCTGCTG
GAPDH-R	ATCTTGGCGAGTGGAGCTAA

Our approach to investigate mechanisms of the innate immune system of pearl oysters is a basis to develop methods to identify stress situations and eventually optimise conditions to prevent disease outbreaks in pearl oyster culture. A critical step in the immune response is the identification of an invading organism as foreign. This recognition step involves interactions between microbial structural motifs and host receptors. This immune recognition process has been carried out by innate immune receptors called PRPs. In oysters, hemocytes are responsible for cell mediated defense. A major defense exhibited by hemocytes involves the direct phagocytosis of antigens. During phagocytosis, the hemocyte recognises and binds to an antigen by the presence of specific lectins either in the hemolymph or in the membrane of the hemocyte (Ford and Tripp, 1996). Though these lectins cannot destroy foreign matter, they are involved in the recognition by different immune modulators leading to its destruction.

In the present study, mRNA level of F-type lectin in the challenged animals increased significantly with respect to the control and reached maximal levels at 4 h and then gradually decreased over time. This may be due to the combined effect of progressive recognition of the bacterial LPS and the clearance of LPS by activating cellular or humoral immune responses. In Japanese sea perch (*Lateolabrax japonicus*), F-type lectin mRNA expression was upregulated at 4 h after LPS stimulation and from the 6 h, the expression level decreased (Qiu *et al*., 2011). In *P. martensii*, the expression level of F-type lectin was significantly increased at 3 h post challenge and then the expression level decreased gradually over time (Chen *et al*., 2011). As shown in Fig. 1, early response of F-type lectin obtained in the study indicated that it is a constitutive and inducible acute-phase protein and maintains the animal in a constant state of readiness by facilitating immediate detection of an invading infectious threat.

Among other PRPs, galectins are important for recognising β-galactoside ligand of the pathogen by their conserved carbohydrate recognition domains (CRD) (Barondes *et al*., 1994) and play crucial roles in innate immunity. Upregulation of invertebrate galectin induced by bacteria, virus, fungi or parasites has been observed in oyster (Zhang *et al*., 2011a), amphioxus (Yu *et al*., 2007) and clam (Kim *et al*., 2008). Lectin–glycan interactions are ubiquitous and essential to biological systems. In bay scallop (*Argopecten irradians*), the mRNA expression level of AiGal2 was upregulated significantly after *Vibrio anguillarum* or *Micrococcus luteus* challenge (Song *et al*., 2011). The present study has indicated that galectin mRNA expression increased significantly with respect to the control and reached maximal levels at 8 h and then dropped down to 36 h. This is in agreement with the report of Zhang *et al.* (2011b) who found that the expression of galectin in *P. fucata* was significantly upregulated at 8 h and 12 h after bacterial challenge. Fermino *et al*. (2011) reported that galectin-3 (Gal 3), glycan binding protein that can be secreted by activated macrophages and mast cells at inflammation sites plays an important role in inflammatory diseases caused by bacteria and their products, such as LPS. Owing to the LPS-galectin-3 interactions during infections caused by Gram negative bacteria, galectin-3 could serve as a sensor to detect small amounts of LPS and allow it to efficiently activate recruited neutrophils. These results suggest that galectin is involved in immune defense against the broad spectrum of bacteria and their products.

LGBP is one important member of the PRPs in invertebrates and displays various biological functions. In scallop *Chlamys farreri,* LGBP gene expression was upregulated initially after stimulation by *V. anguilarum* and subsequently reduced to the normal level (Su *et al*., 2004). Zhang *et al.* (2010) reported that LGBP gene expression was up-regulated at 8 h and 12 h after bacterial and LPS stimulation in *P. fucata*. LPS could significantly upregulate the mRNA level of LGBPs in several marine

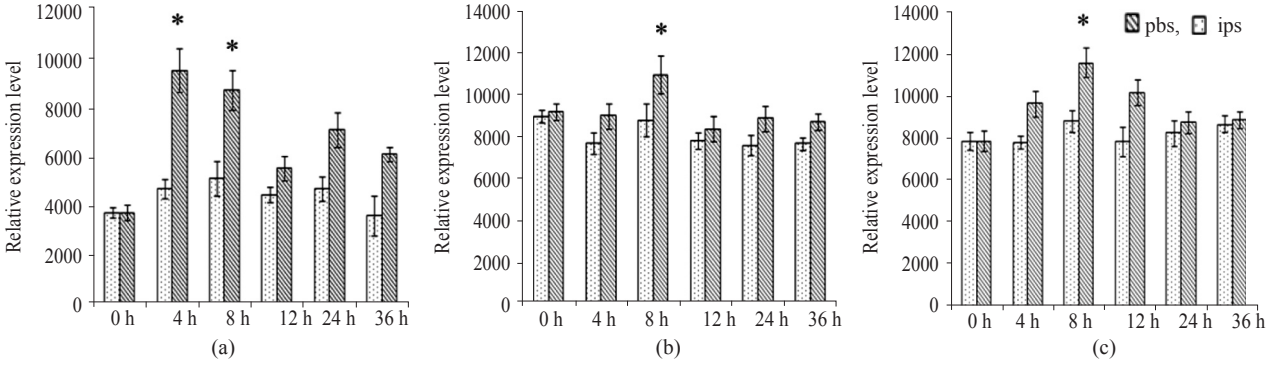

Fig. 1. Expression of PRP mRNA in response to LPS challenge in the pearl oyster, *P. fucata*. (a): F-type lectin, (b): Galectin, (c): LGBP Vertical bars represent the mean±S.E (n=5). Significant differences ($p<0.05$) are indicated with the asterisk (*).

invertebrates, including kuruma shrimp (Lin *et al*., 2008), crayfish (Lee *et al*., 2000) and disk abalone (Nikapitiya *et al*., 2008). In the present study, the gene expression of LGBP was significantly increased at 8 h and then dropped down to the basal level after LPS stimulation.

In conclusion, the PRP genes are not only constitutively expressed genes but can also be induced, enabling them to play critical role in innate immune defense of *P. fucata*. Overall data in the present study revealed that when a foreign object enters the body, the PRP gene shows an increasingly higher level of transcription. At the maximum, the relative mRNA expression of F-type lectin, galectin and LGBP increased to 2.5 fold, 1.5 fold and 1.5 fold respectively, over control. This suggests that PRP functions by recognising PAMP and may activate different immune genes to defend against these pathogens. Hence, information on this PRP in *P. fucata* may be useful in studies of PRPs in other marine invertebrates.

Acknowledgments

This work was financially supported by Indian Council for Agricultural Research (ICAR) and ICAR-Central Marine Fisheries Research Institute (ICAR-CMFRI), Kochi.

Reference

Anju, A., Jeswin, J., Thomas, P. C., Paulton, M. P. and Vijayan, K. K. 2013. Molecular cloning, characterisation and expression analysis of cytoplasmic Cu/Zn-superoxide dismutase (SOD) from pearl oyster *Pinctada fucata*. *Fish Shellfish Immunol*., 34: 946-950.

Barondes, S. H., Castronovo, V., Cooper, D. N., Cummings, R. D., Drickamer, K., Feizi, T., Gitt, M. A., Hirabayashi, J., Hughes, C. and Kasai, K. 1994. Galectins: a family of animal beta-galactoside-binding lectins. *Cell,* 76: 597-598.

Barrionuevo, P., Beigier-Bompadre, M., Ilarregui, J. M., Toscano, M. A., Bianco, G. A., Isturiz, M. A. and Rabinovich, G. A. 2007. A novel function for galectin-1 at the crossroad of innate and adaptive immunity: galectin-1 regulates monocyte/macrophage physiology through a nonapoptotic ERK-dependent pathway. *J. Immunol.,* 178: 436-445.

Cammarata, M., Salerno, G., Parisi, M. G., Benenati, G., Vizzini, A., Vasta, G. R. and Parrinello, N. 2012. Primary structure and opsonic activity of an F-lectin from serum of the gilt head bream *Sparus aurata* (Pisces, Sparidae). *Ital. J. Zool*.,79(1): 34-43.

Chen, H. Y., Sharma, B. B., Yu, L., Zuberi, R., Weng, I. C., Kawakami, Y., Kawakami, T., Hsu, D. K. and Liu, F. T. 2006. Role of galectin-3 in mast cell functions: galectin-3 deficient mast cells exhibit impaired mediator release and defective JNK expression. *J. Immunol.,* 177: 4991-4997.

Chen, J., Xiao, S. and Yu, Z. 2011. F-type lectin involved in defense against bacterial infection in the pearl oyster (*Pinctada martensii*). *Fish Shellfish Immunol.,* 30: 750-754.

Cheng, W., Liu, C. H., Tsai, C. H. and Chen, J. C. 2005. Molecular cloning and characterisation of a pattern recognition molecule, lipopolysaccharide and β-1,3 glucan binding protein (LGBP) from the white shrimp *Litopenaeus vannamei. Fish Shellfish Immunol.,* 18: 297-310.

Fermino, M. L., Polli, C. D., Toledo, K. A., Liu, F. T. and Hsu, D. K. 2011. LPS Induced galectin-3 oligomerisation results in enhancement of neutrophil activation. *PLoS ONE.,* 6(10): e26004. doi:10.1371/journal.pone.002.

Ford, S. E. and Tripp, M. R. 1996. Diseases and defense mechanisms. In: Kennedy, V. S., Newell, R. I. E., Eble, A. F. (Eds.), The eastern oyster: *Crassostrea virginica*. Maryland Sea Grant College, College Park, MD, p. 581- 660.

Gauthier, L., Rossi, B., Roux, F., Termine, E. and Schiff, C. 2002. Galectin-1 is a stromal cell ligand of the pre-B cell receptor (BCR) implicated in synapse formation between pre-B and stromal cells and in pre-BCR triggering. *Proc. Natl. Acad.. Sci.,. USA.,* 99: 13014-13019.

Hernandez, J. D. and Baum, L. G. 2002. Ah, sweet mystery of death! Galectins and control of cell fate. *Glycobiology,* 12: 127-136.

Honda, S., Kashiwagi, M., Miyamoto, K., Takei, Y. and Hirose, S. 2000. Multiplicity, structures, and endocrine and exocrine natures of eel fucose-binding lectins. *J. Biol. Chem.,* 275: 33151-33157.

Hsu, D. K., Yang, R. Y. and Liu, F. T. 2006. Galectins in apoptosis. *Method. Enzymol.,* 417: 256-273.

Iwanaga, S. and Lee, B. L. 2005. Recent advances in the innate immunity of invertebrate animals. *J. Biochem. Mol. Biol.,* 38(2): 128-150.

Kim, Y. S., Ryu, J. H., Han, S. J., Choi, K. H., Nam, K. B. and Jang, I. H. 2000. Gram negative bacteria-binding protein, a pattern recognition receptor for lipopolysaccharide and β1,3-glucan protein that mediates the signaling for the induction of innate immune gene in *Drosophila melanogaster* cells. *J. Biol. Chem.,* 275: 32721-32727.

Kim, J. Y., Kim, Y. M., Cho, S. K., Choi, K. S. and Cho, M. 2008. Noble tandem-repeat galectin of Manila clam *Ruditapes philippinarum* is induced upon infection with the protozoan parasite *Perkinsus olseni*. *Dev. Comp. Immunol*., 32: 1131-1141.

Lee, W. J., Lee, D. J., Kravchenko, V. V., Ulevitch, R. J. and Brey, P. T. 1996. Purification and molecular cloning of an inducible Gram negative bacteria-binding protein from the silkworm *Bombyx mori. Proc.Natl. Acad. Sci. USA,* 93: 7888-7893.

Lee, S. Y., Wang, R. and Soderhall, K. 2000. A lipopolysaccharide and beta-1,3 glucan-binding protein from hemocytes of the freshwater crayfish *Pacifastacus leniusculus*. Purification, characterisation and cDNA cloning. *J. Biol. Chem.,* 275(2): 1337-1343.

Leffler, H., Carlsson, S., Hedlund, M., Qian, Y. and Poirier, F. 2004. Introduction to galectins. *Glycoconj. J.,* 19: 433-440.

Liao, D. I., Kapadia, G., Ahmed, H., Vasta, G. R. and Herzberg, O. 1994. Structure of S-lectin, a developmentally regulated vertebrate-galactoside-binding protein. *Proc. Natl. Acad. Sci. USA,* 91: 1428-1432.

Lin, Y. C., Vaseeharan, B. and Chen, J. C. 2008. Identification and phylogenetic analysis on lipopolysaccharide and b-1,3-glucan binding protein (LGBP) of kuruma shrimp *Marsupenaeus japonicus*. *Dev. Comp. Immunol.,* 32(11): 1260-1269.

Moy, G. W. and Vacquier, V. D. 2008. Bindin genes of the Pacific oyster *Crassostrea gigas*. *Gene,* 423: 215-220.

Naganuma, T., Ogawa, T., Hirabayashi, J., Kasai, K., Kamiya, H. and Muramoto, K. 2006. Isolation, characterisation and molecular evolution of a novel pearl shell lectin from a marine bivalve, *Pteria penguin*. *Mol. Divers.,* 10(4): 607-618.

Nikapitiya, C., De Zoysa, M. and Lee, J. 2008. Molecular characterisation and gene expression analysis of a pattern recognition receptor from disk abalone, *Haliotis discus discus*. *Fish Shellfish Immunol.,* 25: 638-647.

Odom, E. W. and Vasta, G. R. 2006. Characterisation of a binary tandem domain F-type lectin from striped bass (*Morone saxatilis*). *J. Biol. Chem.,* 281: 1698-1713.

Qiu, L., Zhang, H., Jiang, S., Lin, L., Li, J., Yang, K. and Zou, F. 2011. Molecular cloning and expression analysis of a F-type lectin gene from Japanese sea perch (*Lateolabrax japonicus*). *Mol. Biol. Rep.,* 38: 3751-3756.

Rabinovich, G. A., Rubinstein, N. and Toscano, M. A. 2002. Role of galectins in inflammatory and immunomodulatory processes. *Biochem. Biophys. Acta.,* 1572: 274-284.

Raetz, C. R., Ulevitch, R. J., Wright, S. D., Sibley, C. H. and Ding, A. 1991. Gram- negative endotoxin: an extraordinary lipid with profound effects on eukaryotic signal transduction. *FASEB J.,* 5: 2652-2660.

Roux, M. M., Pain, A., Klimpel, K. R. and Dhar, A. K. 2002. The lipopolysaccharide and β-1,3-glucan binding protein gene is upregulated in white spot virus infected shrimp (*Penaeus stylirostris*). *J. Virol.,* 7140-7149.

Salerno, G., Parisi, M. G., Parrinello, D., Benenati, G., Vizzini, A., Vazzana, M., Vasta, G. R. and Cammarata, M. 2009. F-type lectin from the seabass (*Dicentrarchus labrax*): Purification, cDNA cloning, tissue expression and localisation and opsonic activity. *Fish Shellfish Immunol.,* 27: 143-153.

Salto, T., Hstada, M. and Iwangaga, S. 1997. A newly identified horseshoe crab lectin with binding specificity to O-antigen of bacterial lipopolysaccharides. *J. Biol. Chem.,* 272: 30703-30708.

Sharon, N. and Lis, H. 2004. History of lectins: from hemagglutinins to biological recognition molecules. *Glycobiology*, 14: 53-62.

Song, X., Zhang, H., Wang, L., Zhao, J., Mu, C., Song, L., Qiu, L. and Liu, X. 2011. A galectin with quadruple-domain from bay scallop *Argopecten irradians* is involved in innate immune response. *Dev. Comp. Immunol.,* 35: 592-602.

Su, J., Song, L., Xu, W., Wu, L., Li, H. and Xiang, J. 2004. cDNA cloning and mRNA expression of the lipopolysaccharide and beta-1,3-glucan-binding protein gene from scallop *Chlamys farreri. Aquaculture,* 239: 69-80.

Tasumi, S. and Vasta, G. R. 2007. A galectin of unique domain organization from hemocytes of the eastern oyster (*Crassostrea virginica*) is a receptor for the protistan parasite *Perkinsus marinus. J. Immunol.,* 179: 3086-3098.

Toscano, M. A., Ilarregui, J. M., Bianco, G. A., Campagna, L., Croci, D. O., Salatino, M. and Rabinovich, G. A. 2007. Dissecting the pathophysiologic role of endogenous lectins: glycan-binding proteins with cytokine-like activity? *Cytokine Growth Factor Rev.,* 18: 57-71.

Ulevitch, R. J. and Tobias, P. S. Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. *Annu. Rev. Immunol.,* 13: 437-457

Vasta, G. R. and Ahmed, H. 2008. *Animal lectins: a functional view*. Boca Raton, CRC Press, 558 pp.

Vasta, G. R., Ahmed, H. and Odom, E. W. 2004. Structural and functional diversity of lectin repertoires in invertebrates, protochordates and ectothermic vertebrates. *Curr. Opin. Struct.Biol.,* 14: 617-630.

Yamaura, K., Takahashi, K. G. and Suzuki, T. 2008. Identification and tissue expression analysis of C-type lectin and galectin in the pacific oyster, *Crassostrea gigas*. *Comp. Biochem. Physiol. B*., 149: 168-175.

Yoshino, T. P., Dinguirard, N., Kunert, J. and Hokke, C. H. 2008. Molecular and functional characterisation of a tandem-repeat galectin from the freshwater snail *Biomphalaria glabrata*, intermediate host of the human blood fluke *Schistosoma mansoni*. *Gene,* 411: 46-58.

Yu, Y., Yuan, S., Yu, Y., Huang, H., Feng, K., Pan, M., Huang, S., Dong, M., Chen, S. and Xu, A. 2007. Molecular and biochemical characterisation of galectin from amphioxus: primitive galectin of chordates participated in the infection processes. *Glycobiology,* 17: 774-783.

Zhang, D., Ma, J., Jiang, J., Qiu, L., Zhu, C., Su, T., Li, Y., Wu, K. and Jiang, S. 2010. Molecular characterisation and expression analysis of lipopolysaccharide and β-1,3-glucan-binding protein (LGBP) from pearl oyster *Pinctada fucata. Mol. Biol. Rep.,* 37: 3335-3343.

Zhang, D., Jiang, S., Hu, Y., Cui, S., Guo, H., Wu, K., Li, Y. and Su, T. 2011a. A multidomain galectin involved in innate immune response of pearl oyster *Pinctada fucata*. *Dev. Comp. Immunol.,* 35: 1-6.

Zhang, D. C., Hu, Y. T., Guo, H. Y., Cui, S. G., Su, T. F. and Jiang, S. G. 2011b. cDNA cloning and mRNA expression of a tandem-repeat galectin (PoGal2) from the pearl oyster, *Pinctada fucata. Genet. Mol. Res.,* 10 (3): 1963-1974.

Date of Receipt : 19.03.2013
Date of Acceptance : 02.05.2015