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Effect of Salinity Stress on Gene Expression in Black Tiger Shrimp *Penaeus monodon*

Shekhar S. Mudagandur, Gopikrishna Gopalapillay and Koyadan K. Vijayan

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Abstract

Penaeid shrimps are euryhaline in nature and have the ability to survive and adapt in a wide range of salinities (3–50 ppt). The shrimps are cultured under a variety of conditions in many tropical and subtropical countries. Osmotic and ionic regulation is an important mechanism of environmental adaptation in crustaceans. However, drastic changes in abiotic and biotic conditions result in stress to the shrimps during the culture period. Salinity and temperature are the two major environmental factors that have huge impact on culture shrimp, affecting their physiological and metabolic parameters, which in turn affect shrimp growth, molting, and survival. Changes in the abiotic factors, chemical and biotic factors result in reduced immunity of shrimp and vulnerability to bacterial and viral diseases. This chapter describes the effects of low and high salinity on the gene profile changes of black tiger shrimp *Penaeus monodon*, and the functional role of these genes in shrimp salinity stress is discussed.

Keywords: *Penaeus monodon*, Salinity stress, Differentially expressed genes

1. Introduction

Penaeid shrimps being euryhaline can adapt to thrive and survive in a wide range of salinity conditions. Shrimps are, therefore, cultured and reared under different farming conditions in tropical and subtropical countries. Water quality management is an important criterion in shrimp farming for survival and growth of the shrimp. The optimal salinity conditions for penaeid shrimp ranges differently for different species. *Penaeus monodon*, which can tolerate low salinity of 5 ppt to high salinity conditions of 40 ppt has optimal range of salinity (15–25 ppt) for optimal growth [1]; juvenile *Penaeus chinensis* grows best at 20–30 ppt salinity range [2]; the optimal conditions of salinity for growth was estimated to be in the range of 22–34 ppt

for *Penaeus latisulcatus* [3]. *Penaeus semisulcatus*, an Indo-Pacific species requires higher salinity (30–35 ppt) for growth [4]. Best performance (growth, survival, total biomass) for *Penaeus indicus* PL20 and PL60 at salinities between 20 and 30 ppt was observed after acclimation period [5]. Highest increase in biomass and production was observed at 25 ppt for *Penaeus merguiensis* [6]. *Litopenaeus vannamei*, a native species of the Pacific coast is a very important shrimp species, which is cultured under semi-intensive and intensive conditions in many parts of world. The juveniles of this species have optimal growth and survival in salinity range of 33–40 ppt [7]. It is also preferred for culture in low salinity water as it can tolerate very low salinities of 1–2 ppt.

However, the variable climate conditions result in drastic changes in abiotic factors causing stress to the shrimps during the culture period, which influences culture of euryhaline penaeids. In summer months, there is increase in salinity in ponds due to high evaporation rates and in rainy season the salinity decreases. The marine crustaceans are generally osmotic and ionic conformers in nature. The shrimps hypo-osmoregulate above the iso-osmotic point and hyper-osmoregulate below the iso-osmotic point through a osmoregulation mechanism.

In crustaceans, gills which are highly permeable external surfaces are the primary sites involved in osmoregulation. In the larval stages of penaeids, the typical features of osmoregulatory epithelia are present in pleurae and branchiostegites [8]. The overall ion-transport and osmotic regulation process involves ions absorbtion or excretion between the external and internal medium through osmoregulatory organs, such as gills, the antennal glands that mainly function in urine production, and the gut in decapod crustaceans [9-10]

The crustaceans have two well-known important enzymes which are central to osmotic and ionic regulation and ion uptake. The transepithelial movement of monovalent ions requires the action of Na$^+$/K$^+$-ATPase or the sodium pump utilizing ATP as energy source. The other major enzyme involved in osmoregulation is transport-related enzyme carbonic anhydrase, which plays a role in producing H$^+$ and HCO$_3^-$ through catalysis of respiratory CO$_2$, for counterions in Na$^+$ and Cl$^-$ uptake. In penaeids, carbonic anhydrase is reported to be involved in both hyper- and hypo-osmotic regulation and is induced against low and high salinity exposure, indicating its role in ion uptake and excretion process [11].

In our study, we have constructed suppression subtractive hybridization (SSH) cDNA libraries to identify differentially expressed genes in shrimp *P. monodon*, in response to salinity stress. The SSH clones obtained from the forward SSH cDNA libraries (Figure 1) and reverse SSH cDNA libraries (Figure 2) constructed from gut tissues of shrimp exposed to low (3 ppt) and high (55 ppt) salinity on BLAST analysis, revealed several functional categories.

Similarly, we obtained several functional categories of genes from the forward SSH cDNA libraries (Figure 3) and reverse SSH cDNA libraries (Figure 4) constructed from gill tissues of shrimp exposed to low (3 ppt) and high (55 ppt) salinity conditions. These differentially expressed genes were subjected to RT-qPCR for gene expression analysis. Based on our study, we discuss herein in this chapter some of the important genes identified as differentially expressed in response to salinity stress in shrimp.
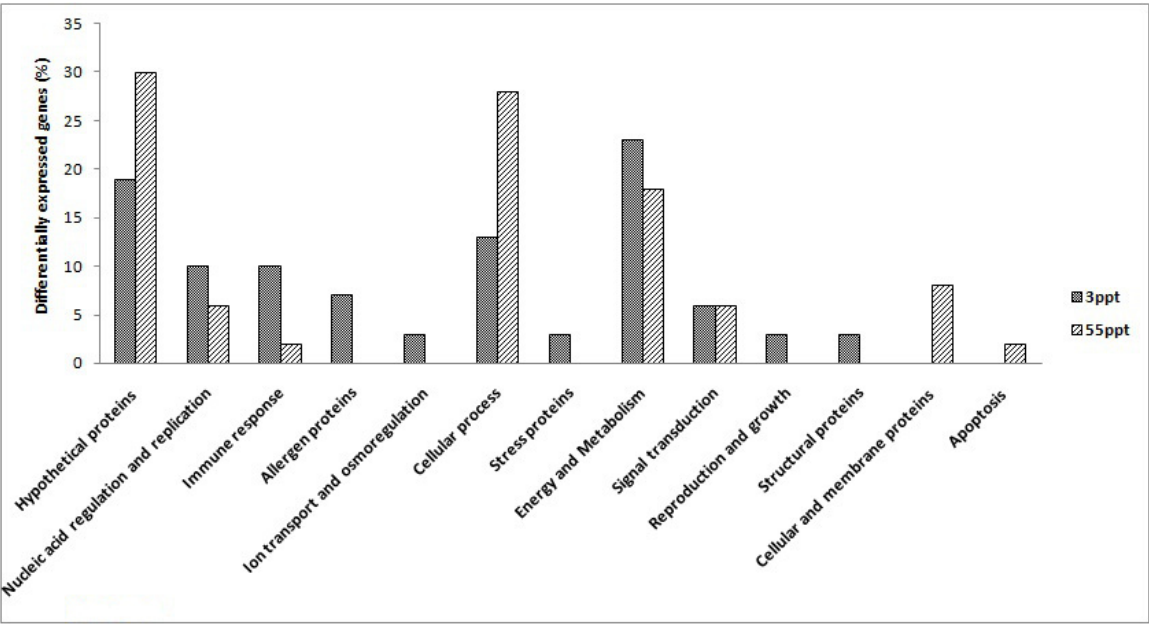
Figure 1. Differentially expressed genes from the forward SSH library of gut tissues of *P. monodon* under low (3 ppt) and high (55 ppt) salinity conditions
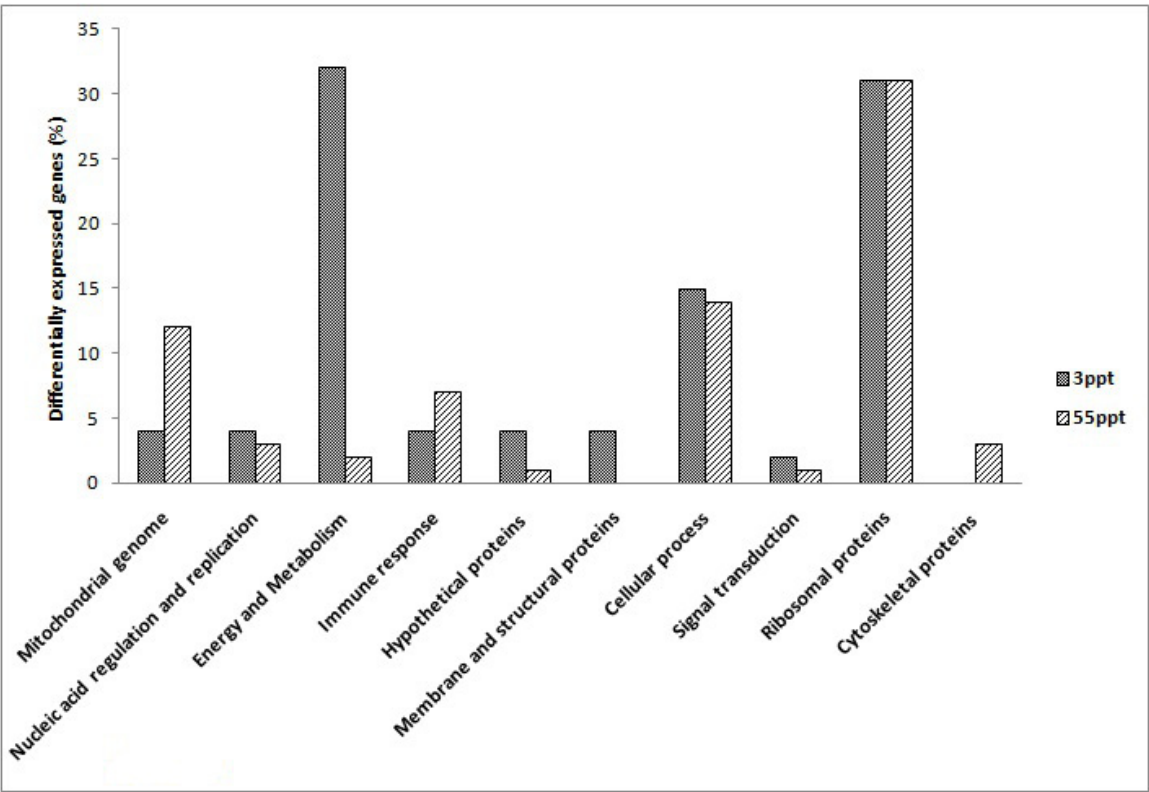

Figure 2. Differentially expressed genes from the reverse SSH library of gut tissues of *P. monodon* under low (3 ppt) and high (55 ppt) salinity conditions
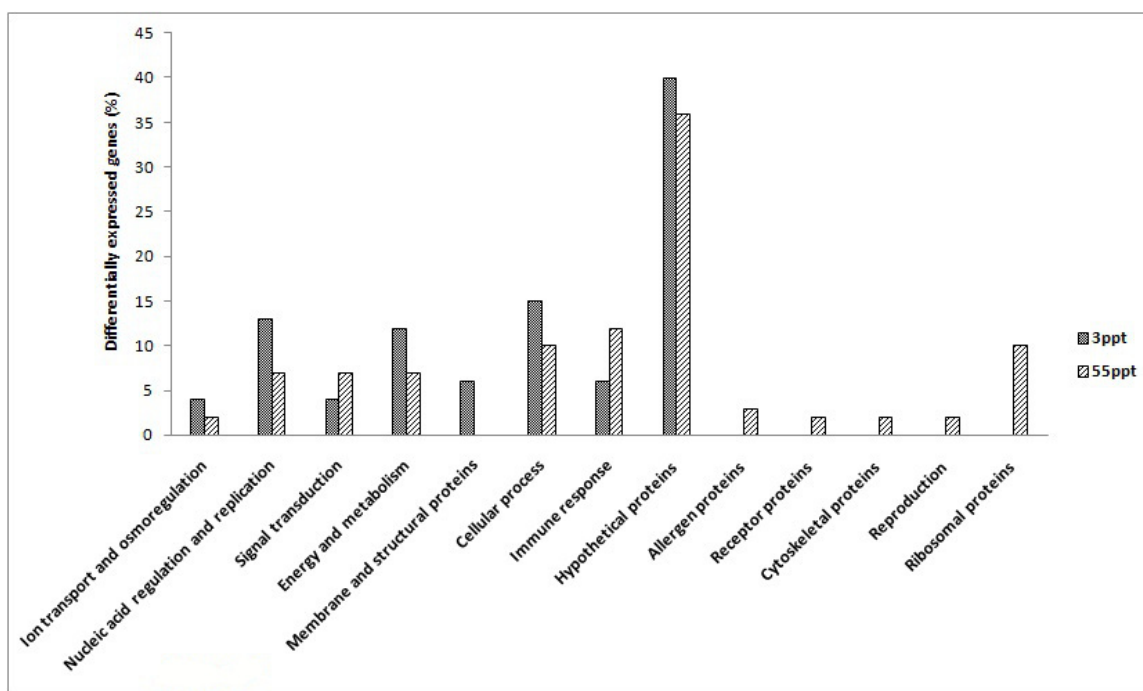
Figure 3. Differentially expressed genes from forward SSH library of gill tissues of *P. monodon* under low (3 ppt) and high (55 ppt) salinity conditions
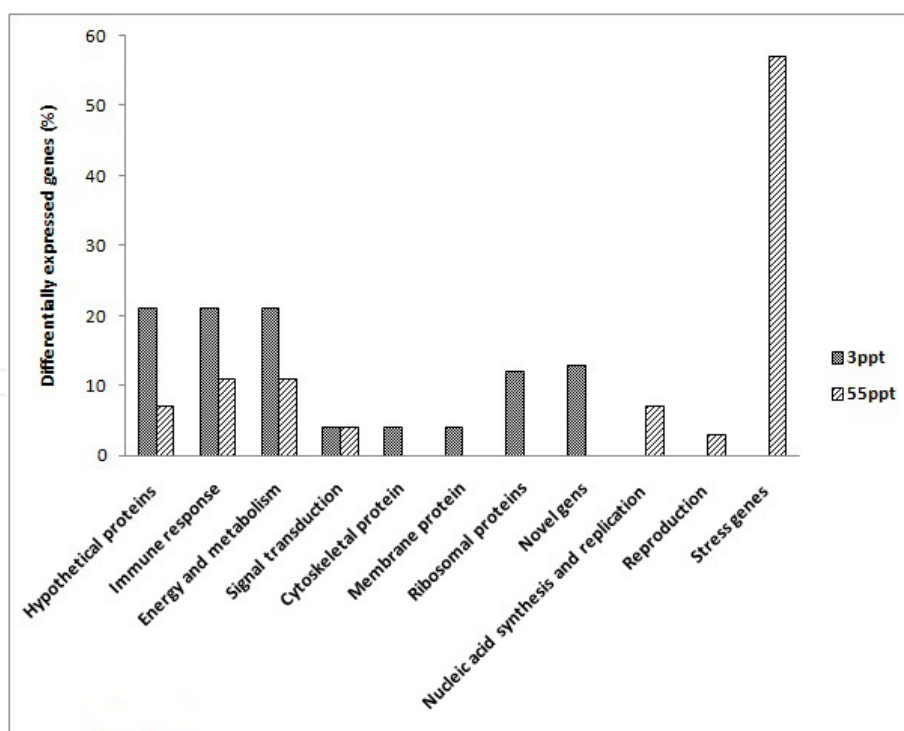

Figure 4. Differentially expressed genes from the reverse SSH library of gill tissues of *P. monodon* under low (3 ppt) and high (55 ppt) salinity conditions

2. Osmoregulatory genes

2.1. Na$^+$/K$^+$-ATPase

Na$^+$/K$^+$-ATPase, a transmembrane protein, contains three subunits, α-, β, and γ- subunit, which are involved in exporting three Na$^+$ from cytosol in exchange for two K$^+$ or NH$_4^+$ from extracellular fluid for each ATP hydrolyzed. The crustacean α-subunit, which is 71–74% identical in amino acid sequence to those of vertebrate α-subunit sequences, binds to ATP and functions for the catalytic action of the enzyme [12]. The binding of Na$^+$/K$^+$-ATPase complex to basolateral membrane requires participation of β-subunit and the γ-subunits of enzyme [13]. The activity of Na$^+$+K$^+$-ATPase in gill tisues of crustaceans depends on the osmoconcentration gradient occurring between hemolymph and the external medium. In crustaceans, there is an increase in Na$^+$/K$^+$-ATPase activity when transferred from natural seawater to dilute seawater [14-15]. With the lowering of salinity when compared to that of normal seawater, the euryhaline crustacea undergo hyperosmoregulation. Increased enzymatic activity of Na$^+$+K$^+$-ATPase and increased α-subunit gene expression has been observed in the gill tissues of crabs [12]. Substantial increase in Na$^+$/K$^+$-ATPase specific activity (300%), Na$^+$/K$^+$-ATPase protein levels (200%), and gene expression level of α-subunit (150%) has been observed in blue crab *Callinectes sapidus* crabs during acclimitization to dilute seawater of 10 ppt salinity [16]. *P. monodon* reared in 7 ppt seawater showed drastic morphological alterations of the antennal glands. The shrimps also showed higher expression and activity of the enzyme Na$^+$/K$^+$-ATPase in the antennal glands under low salinity conditions [17]. *L. vannamei* when transferred to different low salinity conditions ranging from 15 ppt to 1 ppt revealed no significant difference within 3 h for Na$^+$/K$^+$-ATPase α-subunit gene expression and enzyme activity. However, there was a rapid increase at 6 h followed by decrease in the expression level from 12 h to 24 h suggesting Na$^+$/K$^+$-ATPase is stimulated by salinity stress [18]. The study involving V-H ATPase α -subunit and Na$^+$/K$^+$-ATPase β-subunit response to environmental stress (bacteria, pH, Cd, salinity, and low temperature) revealed both the genes to be responsive to these environmental stress conditions. However, the V-H ATPase α -subunit and the Na$^+$/K$^+$-ATPase β-subunit, which is involved in proper folding and transport of Na$^+$/K$^+$-ATPase enzyme, were found to be more sensitive to salinity stress when compared to other stress factors. The exposure of *L. vannamei* to salinity stress resulted in significant changes in the expression of V-H ATPase α-subunit and Na$^+$/K$^+$-ATPase β-subunit gene expression levels in the hepatopancreas and gills of the shrimp. Na$^+$/K$^+$-ATPase β-subunit gene expression after exposure to 5 ppt increased to a highest level (17-fold) at 12 h in the gill tissues, whereas, in hepatopancreas the maximum gene expression levels (4.4-fold) were observed 6 h after exposure to 10 ppt salinity conditions [19].

At low (3 ppt) salinity conditions stress conditions, significant increase in the Na$^+$/K$^+$-ATPase α-subunit gene expression levels was observed in gill (34.28-fold) tissues of *P. monodon* [20]. At higher salinity stress of 55 ppt, *P. monodon* Na$^+$/K$^+$-ATPase gene responded to salinity stress conditions with significant expression levels in gill (15.23-fold) tissues [21]. These results suggests that *P. monodon* Na$^+$/K$^+$-ATPase gene is involved in osmoregulatory process in shrimp and responds significantly under salinity stress.

3. Immune genes

Crustins, belonging to family of antimicrobial peptides (AMPs) are shown to be differentially expressed in response to various immunostimulants and microbes [22]. Different isoforms of crustins have been isolated from a variety of penaeid shrimps [23-25]. The up-regulation of crustin-like AMP in shrimps suggests functional role of AMPs primarily in the shrimp immune response [26]. Under hyperosmotic stress, crustinPm5, crustinPm1, and crustin-like Pm gene levels were found to be up-regulated [27]. In our study, we observed crustin gene was up-regulated in all the three shrimp tissues of gills, gut, and muscle analyzed at low salinity stress [20]. Hence, crustins having antimicrobial activity also functionally respond to salinity stress in shrimps.

Another class of AMPs, the penaeidin gene was found to be down-regulated in gill, gut, and up-regulated in antennal gland tissues of shrimp exposed to high salinity stress (55 ppt) conditions [21]. These changes in the gene expression levels of penaeidin may be regulated through variations in hemocyte numbers in salinity-stressed shrimp. Antibacterial proteins such as lysozymes are involved in nonspecific innate immunity in shrimps [28]. Significant up-regulation of lysozyme gene expression occurred in gill (16.25-fold) tissues of shrimp exposed to low salinity stress [20]. In these shrimps, the anti-lipopolysaccharide factor, which is considered to play an important role in shrimp immune response [29], was significantly down-regulated in gill and gut tissues as compared to up-regulation in the muscle tissues of shrimp.

In general, as a result of environmental stress, the expression of immune-related shrimp genes transcripts gets affected [30]. The shrimp exhibits reduction in immune parameters when exposed to salinity stress conditions, leading to decreased resistance against pathogens [31-32]. White shrimp *L. vannamei*, when transferred to low salinity conditions showed significant decrease in hemocyte count, phenoloxidase activity, respiratory burst, and superoxide dismutase activity, which further reduced on challenge with *Vibrio alginolyticus.* Hence, innate immunity in shrimps reduces with combined effect of low salinity and bacterial challenge [32].

4. Cellular-process-related genes

4.1. Ubiquitin-conjugating enzyme

The ubiquitin proteolytic system is involved in various cellular processes such as cell cycle regulation, cellular response to stress, and immune response [33-34]. In shrimps, the ubiquitin-conjugating enzyme E2r (UBE2r) gene isolated from *Marsupenaeus japonicus* showed that the gene expression level changed significantly in the developing testis and ovary with higher level in the testis than in the ovary, which indicates the functional role of UBE2r in oogenesis and spermatogenesis of shrimp [35]. The gene expression pattern of ubiquitin in abdominal muscle of 3-month-old shrimps *L. vannamei* at different molt stages on examination did not increase significantly in premolt stages and was found to be relatively stable at all stages of

the molt cycle [36]. Antiviral function has been demonstrated with ubiquitin-conjugating enzyme, E2, isolated from *F. chinensis*, which could inhibit WSSV replication and ubiquitinate WSSV RING domain-containing proteins [37]. In *P. monodon* ubiquitin-conjugating enzyme E2, gene was up-regulated in muscle and gill tissues under low salinity stress conditions [20]. In American lobster, *Homarus americanus* the polyubiquitin gene expression revealed signifi‐ cant changes in abdominal muscle and hepatopancreas during osmotic stress, indicating that proteins may be more susceptible to ionic fluctuations [38].

4.2. Cathepsins

The proteases are distributed into four major classes: aspartyl proteases, metalloproteases, serine proteases, and cysteine proteases [39]. The cathepsins belonging to cysteine proteases in addition to cellular protein degradation and turnover are also involved in numerous other physiological processes. In fishes, the cathepsin La isoform has been implicated in yolk‐ processing mechanism during oogenesis and embryogenesis [40]. In shrimps (*Metapenaeus ensis*), cathepsin L, with predominant expression in hepatopancreas, is suggested to have a role in food digestion. The immunolocalization of cathepsin L in the nucleus of oocyte suggests its specific physiological role in shrimp oocytes [41]. The cathepsin L-like proteinases isolated from *L. vannamei* showed that specific activity and mRNA expression of cysteine protease were associated during the molt cycle [42]. The expression of the cathepsin C gene expression in hemocytes of *L. vannamei* could be induced after *V. alginolyticus* challenge, which reached a maximum level at 4 h post challenge period, indicating that the cathepsins in shrimp may be involved in immune response [43]. The aspartic protease cathepsin D obtained from *M. japonicus* and *P. monodon* were characterized for physical and chemical properties and were shown to have identical subunits and similar optimal pH [44]. The analysis of cathepsin B gene expression in *L. vannamei* tissues revealed high expression levels in midgut gland and gut, which are involved in food digestion process. The changes in the gene expression levels and enzymatic activity were induced by starvation in the midgut gland of the white shrimp [45]. Significant increase in the cathepsin B gene expression levels in gills, gut, and muscle tissues of shrimp exposed to low (3 ppt) salinity conditions suggest that *P. monodon* cathepsin gene responds to the shrimp adaptive mechanism to salinity stress [20].

5. Signal transduction genes

5.1. 14-3-3 protein

The members of the 14-3-3 protein family are dimeric proteins that are expressed in a wide range of organisms and tissues. They are involved in modulation of protein interactions through phosphorylation process. The other diverse functional roles include interaction with a large number of protein kinases, DNA, Raf kinase, and regulation of cell cycle progression [46]. In plants, 14-3-3 protein activates and regulates plasma membrane H^+-ATPase through fusicoccin responsive system [47]. Two Na^+/K^+-ATPase α -subunit forms were detected in the gill transcripts of crab *Pachygrapsus marmoratus*, which differed in presence of an 81-nucleotide

sequence near the translation start site in the D form when compared to the C form. The extended D form was found to contain the binding motif for the regulatory protein 14-3-3, suggesting that Na$^+$/K$^+$-ATPase may be stimulated by this regulatory protein binding [48]. The two isoforms of 14-3-3 proteins identified from the *P. monodon* were shown to have varied gene expression profiles during salinity adaptation in response to hypo-osmotic (3 ppt) or hyper-osmotic (40 ppt) salinity stress conditions. Induction of 14-3-3B gene expression in gills of shrimp acclimated to low salinity water suggests that it is likely to be involved in controlling osmoregulation in *P. monodon* under hyperosmotic conditions [49]. The up-regulation of the 14-3-3 gene expression in the tissues of shrimp exposed to low (3 ppt) or high (55 ppt) salinity conditions suggests that *P. monodon* 14-3-3 gene may have a potential role in shrimp response to salinity stress [20-21].

5.2. Calreticulin

Calreticulin, a versatile lectin-like chaperone and important endoplasmic reticulum luminal resident protein, is involved in Ca^{2+} homeostasis and molecular chaperoning. Calreticulins, which are highly conserved in most of the eukaryotes, are involved in the synthesis of various molecules and in many other biological and physiological processes of an organism. The highest expression of calreticulin was detected in ovary of *F. chinensis.* The gene expression varied at different molting stages and could be induced by heat shock and WSSV challenge, indicating multifunctional role of calreticulin [50]. The *P. monodon* calreticulin showed changes in expression profile in response to high-temperature stress, indicating its potential as a biomarker for stress responses in shrimps [51]. Calreticulin also responds to salinity stress with significant increase in the gene expression levels in gills and muscle tissues of *P. monodon* [20].

5.3. Innexins

Innexins, which are members of large multigene families, are involved in formation of gap junctions for cell-to-cell communication [52]. In crustaceans, innexin expression has been associated with developing lobster stomatogastric nervous system [53]. In *P. monodon*, the transcripts of innexin-2-like protein showed increased expression in response to yellow head viral disease [54]. Innexin-2 was found to be more abundantly expressed in testes than ovaries of *P. monodon*, indicating functional role of innexin-2 in spermatogenesis but not in oogenesis [55]. The high gene expression observed for innexin 2 with 14.43-fold in muscle tissue of shrimp under low salinity stress of 3 ppt indicates gap junctions regulation during salinity stress in shrimps [20].

6. Energy and metabolism genes

6.1. Arginine kinase

Arginine kinase plays a major role in energy metabolism and is a phosphotransferase that catalyzes the reversible transfer of phosphate from phosphoguanidine to ADP, resulting in

generation of ATP [56]. In shrimps, Penm2 allergen gene having conserved guanidino specificity region and showing very high sequence similarity with crustacean arginine kinase has been isolated and characterized from *P. monodon* [57]. In crustaceans, such as in blue crab *Callinectes sapidus*, the arginine kinase gene expression is associated with salinity changes. The arginine kinase flux reduced under hyperosmotic treatments and increased with the hypo-osmotic treatments [58]. *M. japonicus* when subjected to severe hypoxic stress revealed up-regulation of arginine kinase indicating metabolic response of arginine kinase under hypoxic stress [59]. Arginine kinase was found to be differentially expressed and up-regulated in WSSV-infected blue shrimp (*Penaeus stylirostris*) [60] and the gene expression could be induced against LPS immunostimulation in *L. vannamei* indicating its correlation with immune response in shrimps [61]. The differential expression of arginine kinase in gills, gut, and muscle tissues of shrimp exposed to low (3 ppt) salinity conditions indicates that arginine kinase plays an important role in metabolic process associated with salinity stress in crustaceans [20].

6.2. Ferritin

Ferritin plays a functional role in iron storage and metabolism. In shrimps, this large multi-functional and multisubunit protein gene has been isolated and characterized from *L. vannamei*, which revealed that ferritin is expressed in most of the tissues of shrimp with major expression in hemocytes [62]. The administration of ferritin resulted in increased immune response in *L. vannamei* with enhanced survival rate in WSSV-challenged shrimps and maintained physiological homeostasis of shrimps [63]. In *M. rosenbergii*, the isolated ferritin gene showed conserved domain for the ferroxidase center and the administration of iron enhanced expression of ferritin gene in a tissue-specific manner [64]. The recombinant ferritin was shown to confer protection in *P. monodon* infected with *Vibrio harveyi* [65]. The expression of ferritin mRNA could be induced with heavy metal ions Cu^{2+} and Zn^{2+} and WSSV challenge in *F. chinensis* [66]. The gill tissues of *P. monodon* when subjected to low (3 ppt) salinity stress revealed significant increase in ferritin gene expression (8.79-fold), indicating its functional role in salinity stress in shrimps [20].

6.3. Intracellular fatty-acid-binding proteins

Intracellular fatty-acid-binding proteins (FABPs) are lipid-binding proteins that help in transport of fatty acids across extra- and intracellular membranes and are involved in various other biological processes such as modulation of signal transduction; gene transcription, especially of lipid metabolism; and cell growth and differentiation [67]. FABPs have been well-characterized in vertebrates as compared to that in invertebrates. In crustaceans, the FABP cDNA having fatty-acid-binding motifs has been cloned and characterized from the freshwater crayfish *Pacifastacus leniusculus* and *P. monodon* [68]. The activity of specific Na^+, K^+, Ca^{2+}, and Cl^- ion channels are regulated by various fatty acids. These ion channel regulations may be carried out directly through fatty acid interactions [69]. The shrimp (*P. monodon*) gut tissue revealed highest gene expression level of FABP (14.05-fold) at high salinity stress conditions (55 ppt) and at low salinity stress conditions (3 ppt) with 13.30-fold; the osmoregulatory process may therefore involve the FABPs in shrimps [20-21].

6.4. Acyl-CoA binding protein

Acyl-CoA binding protein (ACBP) is a highly conserved protein. In yeast it is involved in transportation of acyl-CoA esters from the fatty acid synthetase to acyl-CoA-consuming process [70]. The protein, which was first identified in mammals, acts as a neuropeptide that prevents binding of diazepam/endozepine to GABA receptor system [71-72] and is also involved in regulation of several acyl-CoA-dependent processes [73]. In addition, ACBP is involved in many other functions, which include regulating biosynthesis of fatty acid, functional regulation of enzymes and genes, intracellular acyl-CoA pool regulation, acyl-CoA esters donation required for β-oxidation and vesicular trafficking [74], and in regulation of m-calpain [75]. In plants *Arabidopsis thaliana*, different types of ACBPs are encoded [76], such as the ACBP1 and ACBP2, which are membrane-associated proteins [77-80]; ACBP3, which is the extracellularly targeted protein [81]; and ACBP4, ACBP5, and ACBP6, which are the cytosolic proteins [82-83]. ACBPs are involved in abiotic stress tolerance in plants. The ACBP2 in *Arabidopsis* was shown to be involved in heavy metal (Cd) tolerance [84-85]. ACBP6 and ACBP1 are functionally involved in increased freezing tolerance [78],[86]. In shrimp, ACBP functions in antibacterial [87] and antiviral response [88]. The ACBP gene in *P. monodon* was identified to be differentially expressed in the SSH libraries constructed from the gut tissues of both low (3ppt) and high (55ppt) salinity stressed shrimps. The complete cDNA sequence of ACBP consisted of 273 bp ORF coding for 90 amino and showed ligand-binding conserved domains similar to the other members of ACBP family. At 2 weeks post 3 ppt salinity stress conditions, a significant increase in the ACBP transcripts expression was observed in gills and muscle tissues with highest levels in the gut tissues (28.08-fold). Similar increase in the gene expression levels was observed in shrimps exposed to high salinity stress conditions of 55 ppt in gills and muscle tissues with gut tissues revealing high (11.95-fold) levels of gene expression [89]. These results suggest a functional role of ACBP gene during salinity stress in shrimps.

6.5. Catechol-O-methyltransferase

O-methyltransferase (OMT) is an enzyme found in a wide range of organisms such as microbes [90], where it is involved in antibiotic biosynthesis [91], and in fungi, where it is involved in biosynthesis of aflatoxins [92]. The OMT found in plants are well characterized for their functional role in O-methylation during biosynthesis of lignin, stress resistance, and disease tolerance [93]. In crustaceans, farnesoic acid O-methyltransferase (FAMeT) catalyzes farnesoic acid methylation resulting in production of isoprenoid methyl farnesoate, which is involved in metabolic and physiological regulation [94]. Catechol-O-methyltransferase (COMT), which is a type of O-methyltransferases, helps in catalyzing the transfer of methyl group to the hydroxyl group of catechol compounds from S-adenosyl-L-methionine. In higher animals, the COMT helps in catalysis of methylation of various macromolecules that are involved in different functional and regulatory purposes and is present in soluble and membrane-bound forms [95]. In shrimps, the construction of suppression subtractive hybridization (SSH) libraries from *P. monodon* gill tissues resulted in identification of COMT gene as one of the differentially expressed genes subjected to salinity stress. The COMT gene was differentially regulated in both the SSH libraries generated from low and high salinity conditions. The ORF of COMT gene of 666 bp size revealed the coding protein with 221 amino acids [96]. The *P. monodon* COMT showed the conserved domains present in superfamily of S-adenosylmethio-

nine-dependent methyltransferases, which includes COMT, CCoAOMT family of indolethyl-amine N-methyltransferase from humans, and OMT from *Bacillus subtilis* [97]. The *P. monodon* COMT was found to be up-regulated in low and high salinity stress conditions at different time intervals in shrimp tissues (gills, guts, and muscles), suggesting a functional role of this gene in salinity stress tolerance in shrimps [96].

7. Stress genes

7.1. Heat shock proteins

Heat shock proteins (HSPs) initially discovered in *Drosophila melanogaster* are a highly conserved set of polypeptides present in both prokaryotic and eukaryotic cells. They are generally involved in conferring thermotolerance as molecular chaperones by refolding the denatured proteins and also respond against various other stresses. They play a crucial role in organisms' response to heat shock and cellular stress. In addition, the HSPs are also important for cellular damage protection and in maintaining cellular homeostasis [98]. In aquatic animals, the HSPs respond to environmental pollutants, abiotic stress, and are involved in disease resistance against viral and bacterial pathogens. The functional role and significance of HSP in farmed aquatic animals is demonstrated in stimulating the immune response [99]. In shrimp (*P. monodon*), some of the HSPs such as HSP21 have been characterized for gene expression against WSSV infection [100]; HSP70 gene expression was found to increase in the shrimp hemocytes after heat shock treatment [101]; and HSP90 gene expression has been related with the ovarian maturation [102]. The transcriptional levels of HSP21, HSP70, and HSP90 were inducible under the heat shock and responded upon bacterial exposure in *P. monodon* [103]. Expression of HSP70, which is one of the widely studied HSP in aquatic organisms, was high during short-term hyperthermic treatment when compared to hypoxic and osmotic stress in *P. monodon* [104]. The significant increase in gene expression level HSP21 in the gut and muscle tissues of *P. monodon* exposed to low salinity stress (3 ppt) conditions, indicates its functional role in osmotic stress in shrimps [20].

In conclusion, the construction of SSH cDNA library in response to low (3 ppt) and high (55 ppt) salinity stress in shrimp (*P. monodon*) led to identification of various differentially expressed genes. The significant up-regulation expression of several genes at transcription level in gills, gut, antennal gland, and muscle tissues of shrimp in response to two-week post-salinity stress condition indicates their functional role in gene pathways and regulatory mechanism of osmotic stress at the molecular level.

Acknowledgements

Author details

Shekhar S. Mudagandur[*], Gopikrishna Gopalapillay and Koyadan K. Vijayan

*Address all correspondence to: msshekhar@ciba.res.in

Central Institute of Brackishwater Aquaculture, Chennai, India

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