CHAPTER IV

DISCUSSION

4.1 Tissues for identification of genes controlling osmorality in *P. monodon*

Osmoregulation is one of the most important adaptive physiological processes permitting the successful establishment of a species in a given habitat. (Haond et al., 1998). There are several organs involved in crustacean osmoregulation. In this study, gill, epipodite and antennal gland were used to investigate genes associated with osmoregulatory system in *P. monodon*. Gill of crustaceans not only plays a major role in oxygen consumption but is also being the primary organ for salt transport to maintain salt and water balance (Lucu and Devescovi, 1999; Lucu and Towle, 2003). In some freshwater crustacean species, the antennal urinary glands are involved in osmoregulation through the production of dilute urine (Mantel and Farmer 1983; Pequeux, 1995). The function of the antennal gland in penaeid shrimp has been associated with sodium and chloride concentration in haemolymph. Epipodites are lamellar organs associated with the podobranchs (except on the first maxilliped). Ultrastructural characteristics indicate that epipodite is composed of osmoregulatory tissue (Martinez et al., 2005). The cytoplasm of the epithelial cells of epipodite contains numerous elongated mitochondria indicating strongly the presence of the Na^+/K^+-ATPase. These are characteristics of cells playing a role in osmoregulation and also in other ionic exchange and acid-base regulation. The tissues to be used in this study were osmoregulatory organs which should be potential sources of identifying genes controlling osmoregulation.

4.2 Identification of functionally relevant genes controlling osmorality in *P. monodon* by using Differential Display PCR (DD-PCR)

The Differential Display PCR (DD-PCR) technique was first described by Liang and Pardee (Liang and Pardee, 1992). The strategy to detect differentially expressed genes is based on the fact that when PCR is performed on a complex cDNA sample using one or two arbitrary primers, a reproducible fingerprint of the template is produced. Differences in these fingerprints are due to differences in the template material. DD-PCR method does not depend on the prior knowledge of sequence and availability of cDNA clones. Numerous genes, including candidate tumor suppressor genes, genes regulating cell development, differentiation and many others, have been identified with this widely used DD-PCR technique (Liang and Pardee, 1992; McClelland et al., 1995; Tugores and Belmonte, 1999; Vieites et al., 2005; Walters and Rouvie?re, 2006; Pathak and Kanungo, 2007).

In this study, the DD-PCR profiles were generated from 26 primer combinations using cDNAs of antennal gland, epipodite and gill from the control and stressed shrimp as templates for PCR reaction. Theoretically, each arbitrary 13-mer hybridizes with 7 bases to provide specificity, representing 1/16,000 possible sequences, a given mRNA 3' end sequence of 600 bp resolved on a denaturing polyacrylamide gel contains about 600 sites for recognition by all possible 16,000. Thus, each arbitrary primer will have a 600/ 16,000 = 4% probability of detecting any given mRNA (Liang, 1998). Therefore, in theory, 26 primer combinations (6 arbitrary primers in combination with 6 anchored oligo dT primers) could detect 22% (1-$(0.96)^n$ where n is the number of arbitrary primers) of the expressed genes. Twenty six combinations of primer were selected because they showed higher number of DD-PCR products. With a higher number of DD-PCR products, it became possible to identify more expressed genes.

Three time course experiments were carried out to determine how rapidly changes in the mRNA population occurred following the onset of a salt treatment. The

approximate size of differentially expressed bands ranging from 200 to 1,500 bp was selected. Short sequence is predominantly uninformative for gene classification and prediction of function (Jurecic and Belmont, 2000). Increase product size results in longer sequences that can demonstrate homology to sequences reported in the database (Jurecic et al., 1998). DD-PCR profiles of control and stressed shrimp were compared for each combination primer. Seventeen primer combinations demonstrated 97 differentially expressed bands. DD-PCR patterns from gill produced the highest number of DD-PCR products comparing to those from epipodite and antennal gland.

According to the DD-PCR reaction, about 78 % of differential display bands were the mixture of products of similar size obtained from one cDNA band (Mathieu-Daude et al., 1996). These might be arisen from the co-migration of other non-homologous cDNAs or a technical error while excising bands from the gel. Some of them should be the artifacts that might result in error of identification of differential display cDNAs. In this study, this problem is addressed by sequencing between three to six individual clones per cDNA band. A total of 506 clones of 97 differentially expressed bands were sequenced. After sequence analysis, a total of 129 unique sequences were found. Homology search showed 37 different sequences significantly matched the GenBank database entries whereas 92 different sequences did not significantly matched to any sequences in the GenBank database. The high number of unknown sequences was determined. This may be due to a limited number of sequences is available for shrimp in the GenBank database, even many EST projects have been conducted in many shrimp species (Gross et al., 2001; Rojtinnakorn et al., 2002; Supungul, 2002).

The cDNA fragments identified from DD-PCR as potentially regulated by salinity were chosen to confirm their differential expression by RT-PCR. The criteria to determine which cDNA fragments were considered to be potentially regulated by salinity were the level of differential expression and putative function of those cDNA fragments. Twenty-three of the 129 unique sequences originally identified from DD-PCR were considered to be potentially regulated by salinity stress. Theses sequences showed similarity to carbonic anhydrase, corin isoform 2, sarcolemmal associated

protein 2, NIMA-family kinase Nek 7, karyopherin alpha 4, vacuolar protein sorting 18, CHK1 checkpoint, Rps 16 protein, integrin alpha, 5 hypothetical proteins and 9 unknown gene products. Of the 23 cDNA fragments isolated that were preferentially expressed: sixteen cDNA fragments were from one tissue; six were from two tissues; one was from all studied tissues. This may be postulated that their individual expression were under an organ-specific manner.

4.3 Analysis of expression by semi-quantitative reverse transcription polymerase chain reaction (RT-PCR)

4.3.1 RT-PCR technique for analysis of gene expression

The sensitive nature of PCR may cause the false positives of DD-PCR results. Because the amount of product synthesized increases exponentially during PCR, small differences in quality or purity of the template and pipetting errors have large effects on amplification efficiency and produce large apparent differences in the yield of PCR products among samples. Moreover, the random primers and low-stringency cycling were generally used in DD-PCR analysis. The major limitation of the random primers and low-stringency cycling is imperfect match and high background. Another limitation is nonreproducibility due to imperfect annealing of primer (Sturtevant, 2000). In addition, staining procedure can generate false positive results. In this study, we used silver staining technique to visualize the amplified transcripts of mRNA. This method is feasible and sensitive enough to visualize the amplified products of mRNA differential display (Lohmann et al., 1995). Because of its simplicity and time-saving ability, silver staining has been successfully used to detect the products such as, RAPD (Hu et al., 1997) DNA amplified fingerprinting (Caetano-Anolles and Bassam, 1993) and mRNA differential display.(Xin et al., 1999). However, silver staining can be difficult to balance between too faint and too dark patterns. This method might be occurred an error in identification of differentially expressed bands.

The major obstacle reported for DD-PCR technique is the high incidence of false positives (Liang et al., 1993) thus the differential fragments obtained by DD-

PCR should be confirmed. A variety of methods are used to quantify mRNA expression. In this study, semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) was used to confirm DD-PCR results. Sequences of interested cDNA fragments from DD-PCR analysis were used to design specific primers for RT-PCR analysis. β-actin gene was use as an internal control for RT-PCR. The first stranded cDNA of the confirmation experiment was synthesized from total RNA of different groups of shrimp that used in DD-PCR experiment.

Molting occurs in cycles and involves the shedding of the hard exoskeleton to expose a soft new shell. Crustacean molting cycles are controlled by several mechanisms (Watanabe et al., 2000). Shrimp that used in DD-PCR and RT-PCR analysis was determined for their molting stage. Only shrimp in intermolt stage was used because many genes involved in osmoregulation may change their expression throughout the molting stages. During the molt of crustacean, the uptake of water and ion are stimulated. Many inorganic and organic materials such as protein, calcium and carbonate are necessary to synthesize the cuticle of crustacean species. The uptake of water from the animal immediate surroundings causing the new exoskeleton to expand and hardening, respectively. Molting process was regulated by many genes such as farnesoic acid O-methyltransferase (FaMeT), cryptocyanin, metallothionein, crustacean hyperglycemic hormone (CHH), and carbonic anhydrase. Crustacean hyperglycemic hormone (CHH), and carbonic anhydrase genes were reported to be involved in osmoregulation of crustaceans (Henry, 1996; Lucu and Towle, 2003)

Semi-quantitative RT-PCR is a highly sensitive and specific method useful for the detection of rare transcripts or the analysis of samples available in limiting amount (Carding et al., 1992). RT-PCR assumes that the experimental conditions do not alter the expression levels of the selected control RNA. To quantify and normalize the level of mRNA expression of a target gene, it is necessary to determine the mRNA expression of an endogenous control gene exposed to the same experimental conditions as the target gene. The PCR products of the interesting genes are analyzed relatively to the internal control. However, semi-quantitative RT-PCR is only able to

indicate that one transcript is expressed at a higher level than the other. Housekeeping genes involved in basic functions needed for the sustenance of the cell. These genes are constitutively expressed. Several housekeeping genes are used by an internal control such as glyceraldehydes-3-phosphate dehydrogenase (G3PDH or GAPDH), actins, cyclophilin, 18S rRNA, 28S rRNA and elongation factor-1 alpha (EF-1 alpha).

It is important to select the appropriate conditions of RT-PCR so that the amplification product is clearly visible on an agarose gel and can be quantified. $MgCl_2$ is an essential factor for the *Taq* polymerase to function. Low concentration of $MgCl_2$ may cause low PCR products whereas too high concentration may cause non-specific amplification. Each PCR primer pair performs well in a reaction at a certain annealing temperature so this parameter need to be determine. Most frequently the PCR reaction requires a higher annealing temperature to maintain specificity. For amplification cycle, it is well known that amplification is initially exponential but reaches a plateau when the activity of the enzyme declines or any of the reagents become limiting in the reaction. At plateau, RNAs initially present at different levels may produce the equal intensity of PCR products. Therefore, the number of cycles for amplification needs to determine. In this study, PCR conditions including primer concentration, $MgCl_2$ concentration, template concentration, PCR cycle and annealing temperature were optimized. The β-actin was considered as the internal control for semi-quantitative RT-PCR assay. Its expression appeared relatively stable during salinity stressed (Marone et al., 2001).

4.3.2 Expression analysis of interested cDNA fragments and the possible function in osmoregulation under salinity stress

For conformation by RT-PCR, 23 cDNA fragments were tested in the particular tissues which were observed the differential expression from the DD-PCR analysis. The results confirmed that the relative expression of 21 cDNA fragments was regulated by salinity stress. These 21 cDNA fragments showed similarity to carbonic anhydrase, corin isoform 2, NIMA-family kinase Nek 7, karyopherin alpha 4, vacuolar protein sorting 18, CHK1 checkpoint, Rps 16 protein, integrin alpha, 5

hypothetical proteins and 8 unknown gene products. Two cDNA fragments encoding for sarcolemmal associated protein-2 (S34) and unknown gene (S111) showed no significant different expression in gill and antennal gland of shrimp acclimated at both 3 and 40 ppt salinities.

Although histological and ultrastructural studies of tissues and organs involved in osmoregulation are numerous, the knowledge and information about genes involved in this mechanism of crustacean species is lacking. In previous study, a few genes appear to play a role in osmoregulation. In crustacean, the Na^+/K^+-ATPase and carbonic anhydrase have been shown to play a major role in active transport of ion by various epithelia at transcription and translation levels (Bottcher et al., 1990; Bouaricha et al., 1991; Lopez Mananes et al., 2000).

Carbonic anhydrase

From DD-PCR analysis, S8 unique sequence that identified from epipodite and gill was matched with carbonic anhydrase (Acc. No.EAT43649.1). The trend of expression pattern of the fragment was down-regulated at early time points and up-regulated at late time points in gill of shrimp reared at 3 ppt salinity and in epipodite of shrimp reared at 40 ppt salinity. At 3 ppt salinity stress, the relative expression in shrimp epipodite was down-regulated at both early and late time points. At 40 ppt salinity stress, the relative expression in shrimp gill was unchanged at 6 h, significantly increased at 24 h and finally decreased at 2 weeks. Carbonic anhydrase (CA) catalyzed the reversible hydration of CO_2 and H_2O to HCO^{3-} and H^+. CA has been believed to play a role in active ion uptake, especially across the gill and epipodite of crustaceans (Lopez Mananes et al., 2000; Martinez et al., 2005). CA is present in high activities in the organs responsible for osmotic and ionic regulation (Henry, 1996; Henry, 2001). Physiological studies using isolated perfused gills of crabs indicated that CA supports CO_2 excretion (Henry et al., 2002). CA activity in euryhaline crabs is salinity-sensitive, being induced in the gills of animals transferred to low salinity. There is a ten-fold increase of CA activity in the posterior gills of both *Callinectes sapidus* and *Callinectes similis* in low salinity (Piller et al., 1995). In this

study, the expression at transcription level was also induced in shrimp gill when transferred to low salinity at late time point.

Homology with previously described proteins may be a clue to postulate the possible biochemical roles of these sequences in salt stress response. The cDNA unique fragments discussed below have not been reported to be involved in osmoregulation in marine animals. However, some of them previously reported to be involved in salt stress or ion transport in various organisms such as human, insect and plant.

Corin isoform 2

The S14 unique sequence was matched with the corin isoform 2 (Acc. No.XP_001153181.1). This unique sequence was used to design P-S14 primers for RT-PCR analysis. This gene was isolated from gill. Semi-quantitative RT-PCR analysis at 40 ppt salinity stress revealed that the relative expression levels were significantly down-regulated at 6 h, followed by up-regulated expression at 24 h and reached the similar level as the control group (25 ppt salinity) at late time point (2 weeks). No significant difference in expression was found at 3 ppt salinity. Corin, a type II transmembrane serine protease has been identified as an important enzyme for the production of cardiac hormones. Most of the type II transmembrane serine proteases were identified recently; these proteases are expected to play a role in diverse biological processes. Human corin has recently been shown to activate pro-atrial natriuretic peptide (ANP), a cardiac hormone that reduces high blood pressure by promoting salt excretion, decreasing blood volume, and relaxing vessel tension in a receptor dependent manner (Inagami, 1989; Brenner et al., 1990; Hooper et al., 2000).

Karyopherin (importin) alpha 4

From DD-PCR analysis, S22 unique sequence that identified from antennal gland and epipodite was matched with karyopherin (importin) alpha 4 (Acc. No. XP_975030.1). This unique sequence used to design P-S22 primers. The trend of

expression pattern of this cDNA fragment was significantly increased in shrimp antennal gland at both 3 and 40 ppt salinity stress. In shrimp epipodite, the expression level was decreased at both 3 and 40 ppt salinities except at 2 weeks of 40 ppt salinity, the expression of this cDNA fragment reached the similar level as the control shrimp. Karyopherins are heteromeric molecules composed two major types of components, α- karyopherins and β- karyopherins. It is one of the important genes involved in the transport between cytoplasm and nucleus, that function together to transport molecules through the nuclear complex. There are at least 20 different human karyopherins, each responsible for the transport of a different class of molecule (Conti and Izaurralde, 2001), resulting in considerable diversity in nucleocytoplasmic transport pathways. Generally, karyopherins bind to transport signals on protein or RNA cargo molecules. Several proteins such as RAN GTP-binding protein and cellular apoptosis susceptibility protein bind to karyopherins and participate in the transport process (Fradin et al., 2005; Lee et al., 2005)

Vacuolar protein sorting 18

The S38 unique sequence that was significantly matched with vacuolar protein sorting 18 (Acc. No. XP_974055.1) was used to design P-S38 primers for RT-PCR analysis. The relative expression of this cDNA fragment in shrimp epipodite was found to be down-regulated at low salinity stress when compared with the control. At high salinity stress, the expression was unchanged at 6 h, up-regulated at 24 h and finally reached the similar level as the control shrimp (25 ppt salinity) at late time point (2 weeks). Vacuolar protein sorting (Vps) proteins are known to function in protein transport and vacuole formation. Vps18 is a member of Class C Vps genes that are required for the multiple vesicle transport pathways (Raymond et al., 1992). Homologues of the Class C Vps proteins were identified in multiple organisms, including *Drosophila melanogaster*, *Arabidopsis thaliana*, and human. The Class C Vps homologues in mammals and *Drosophila* were found to form a large oligomeric complex that may be required for membrane trafficking to lysosomes or lysosome-like pigment granules in *Drosophila* eyes. The vacuole (lysosome) is an acidic

compartment containing a variety of hydrolytic enzymes. The functions include degradation of cellular proteins for the recycling of amino acids and storage of cellular metabolites such as amino acids, phosphate, and metal ions. A functional vacuole is necessary for cytoplasmic homeostasis, and several *Saccharomyces cerevisiae* mutants lacking an intact vacuole are sensitive to external pH and osmotic stress (Geienhoner et al., 2001).

Integrin alpha

From DD-PCR analysis, S21 unique sequence that matched with integrin alpha (Acc. No. EAT34371.1) was used to design P-S21 primers. This cDNA fragment was found only in gill. RT-PCR analysis revealed that at 3 ppt salinity stress, the relative expression level of this cDNA fragment was down-regulated at early time point and reached the similar level of control group at 2 weeks. At 40 ppt salinity, the trend of expression pattern was up-regulated expression. Integrins comprise a large family of cell surface receptors that are found in many animal species, ranging from sponges to mammals. Integrins are composed of two subunits, α, β and αβ combination has its own binding specificity and signaling properties. Integrin plays a role in the attachment of a cell to the extracellular matrix (ECM) and to other cells, and in signal transduction from the ECM to the cell (Brakebusch et al., 2000; Brakebusch and Fassler, 2005). Conversely, individual matrix proteins, such as fibronectin, laminins, collagens, and vitronectin, bind to several integrins. (Filippo and Erkki, 1999). Moreover, integrins have recently been identified as playing a role in osmoregulation and regulation of ion movement across cell membranes. A number of groups have demonstrated that integrins regulate potassium, calcium, or chloride fluxes either indirectly (Sheikh-Hamad et al., 1997; Davis et al., 2002; Browe and Baumgarten, 2003; Haussinger et al., 2003; Ritter et al., 2003; Kawasaki et al., 2004) or directly (Browe and Baumgarten, 2003; Ritter et al., 2003; Shakibaei and Mobasheri, 2003; Vom Dahl et al., 2003). Moreover, the ion channel of endothelial cell from bovine pulmonary artery in mammalian is regulated by integrin activity. This regulation use potassium and chloride ions for maintaining membrane potential of this ion channel (Kawasaki et al., 2004).

Rps16 protein

The S30 unique sequence was matched with Rps16 protein (Acc. No.AAH82286.1). The sequences were used to design P-S30 primers. This cDNA fragment was identified from shrimp antennal gland. Semi-quantitative RT-PCR analysis revealed that up-regulation of the cDNA fragment encoding for Rps 16 protein was observed in shrimp antennal gland at both high and low salinity stress. A ribosomal protein is one of many proteins that conjunction with rRNA involved in the cellular process of translation. In plant, one of the important functions of ribosomal protein is resistant of changing soil salinity. Plants respond to salt stress by a variety of physiological, biochemical and molecular changes that enable plants to survive such less than favorable conditions (Hasegawa et al., 2000). Induction of ribosomal genes has been noted to be important during salt stress. Active conservation of the polyribosome, Rps14, 16 and Rpsl23 during desiccation has been associated with high level stress tolerance in plants (Sahi et al., 2006a; Sahi et al., 2006b).

CHK1 checkpoint homolog

The S13 unique sequence that was significantly matched with CHK1 checkpoint homolog (Acc. No.CAJ83813.1) was used to design P-S13 primers. From RT-PCR analysis, the relative expression in gill was significantly increased at all time points for 3 and 40 ppt salinity stress. Checkpoint kinase 1 (CHK1), plays a critical role in the prevention of premature mitosis. Generally, CHK1 is phosphorylated in response to stall replication. CHK1 is required for normal cell proliferation and generally activated in response to UV and agents that stalled DNA replication forks. Basically, high NaCl inhibits the activity of key components of the DNA damage response such as CHK1. In previous study, CHK1 of mammalian renal inner medullary cells was determined for adaptation to high NaCl. After several hours, the cells adapt and begin proliferating again, despite the continued presense of high NaCl. Therefore, CHK1, become increased, phosphorylated and DNA breaks are repaired within a few hours (Dmitrieva and Burg, 2004).

NIMA-family kinase Nek7

The S25 unique sequence was matched significantly with NIMA-family kinase Nek7 (Acc. No. AAT45117.1). The sequence of S25 were used to design P-S25 primers for RT-PCR analysis. This cDNA fragment was discovered only in gill. The trend of expression pattern was significantly increased at high salinity stress whereas, significant decrease in expression was observed at low salinity stress. NIMA is the protein product of the *nimA* gene of the filamentous fungus *Aspergillus nidulans*, required for progression of cells from G_2 into mitosis. NIMA belonged to the family of serine/threonine protein kinase (Kun Ping et al., 1993). NIMA is tightly cell cycle regulated both at the level of transcription (Osmani et al., 1991) and by posttranslational mechanisms, including phosphorylation (Ye et al., 1995) and proteolysis (Pu and Osmani, 1995; Ye et al., 1998). A previous study demonstrated that activation of these kinase was strongly inhibited by various salts. NIMA kinase activity of *Aspergillus nidulans* was progressively inhibited by increasing concentrations of LiBr, NaCl, or KCl. Therefore, the salts may interact preferentially with the enzyme and may be involved in disturbing the subunit interaction in the oligomeric form of NIMA that required for optimal kinase activity (Kun Ping et al., 1993).

Salt-stress response was previously shown to encompass large number of genes. These genes were linked to different pathways and processes such as stress perception and signaling, molecular transportation, biochemical processes, physiological and morphological adaptations. Different stress-regulated genes may have cumulative or exclusive roles in salt tolerance. In this study, the transcripts of cDNA fragments regulated by salt stress belong to a variety of function. For all of the isolated, only carbonic anhydrase has been reported to be involved in osmoregulation in crustacean species. The carbonic anhydrase believed to play a role in active ion uptake, especially across the gills of crustaceans. However, some of the cDNA fragments identified as salinity responsive genes shared homology with known genes enabling their functional annotation. The predicted proteins encoded by the karyopherin (importin) alpha 4, vacuolar sorting protein 18, corin isoform 2, Rps16

and integrin have been reported to be associated with salinity stress for control homeostssis of cells in other non-crustacean organisms. The cDNA encoding for karyopherin and vacuolar sorting proitein 18 identified in this study may function as transporter of essential materials which important for osmotic regulation. The cDNA encoding for Rps 16 showed up-regulation in both high and low salinity stress. It may be postulated to have a role in salt-stress response by regulation of translational machinery. The up-regulation of CHK1 may be resulted from the effect of salt stress which causes DNA breaks.

There were several cDNA fragments demonstrating changes in expression during salinity stress showed similarity to genes of unknown function. In this study, RT-PCR analysis revealed that 8 of the 21 salinity responsive cDNA fragments were similar to genes of unknown function. To predict protein functions of these unknown genes, domain prediction using computer program was performed. Amino sequences of these unknown genes were analyzed by SMART program and Kyte-Doolittle hydrophobicity plots to predict domain protein. Kyte-Doolittle hydrophobicity plots are a rather popular method for detecting hydrophobic regions, and therefore possible transmembrane segments. The plots are designed to display the distribution of polar and non-polar residues along a protein sequences (Kyte and Doolittle, 1982). Amino sequences of S5, S114 and S126 cDNA fragments had potential to be a transmembrane region. Transmembrane region are composed of transmembrane protein, which reside and operate typically within a cell's plasma membrane, but also in the membrane of some subcellular compartments or organelles. Transmembrane region may play a role in ion channel or protein-lined pore. Most transmembrane proteins have hydrophobic regions which span the hydrophobic core of the membrane bi-layer and hydrophilic regions located on the outside or the inside of the membrane (Jayasinghe et al., 2001; Krogh et al., 2001; Fariselli et al., 2003). The feature of transmembrane domains that unites them is their overwhelming aversion to water (hydrophobicity). When a number of hydrophobic amino acids in a sequence cluster together, they can form a segment that would rather hide within the water-free cell-membrane than expose themselves to the water inside and outside of the cell. They can abide water if they have to, but would much rather avoid it altogether. If the

protein has only one such segment, then it likely attaches to membranes in the cell until it is secreted. On the other hand, if it has six or seven, then it may form a channel through the cell membrane for smaller molecules (Claverie and Notredame, 2003).

There were 2 cDNA fragments showed no response to salinity stress at both high and low salinity stress from RT-PCR analysis. The 2 cDNA fragments showed similarity to sarcolemmal associated protein-2, Acc. EAT46394.1 (S34) and unknown gene (S111). The name sarcolemma or sarcolemmal membrane is used to describe the cell membrane of a muscle fiber or muscle cell. The membrane is designed to receive and conduct stimuli. Previous study demonstrated that many transport proteins were located mainly in the sarcolemma and activated during muscle contraction or expression (Hayashi et al., 2000; Nielsen et al., 2003). Some substances such as fatty acid and amino acid taurine are transported across the sarcolemma (Warskulat et al., 2004).

In summary, using DD-PCR and RT-PCR techniques, we identified several differentially expressed genes in response to low (3 ppt) and high (40 ppt) salinity stress. Putative gene homologues that showed highly regulated by salinity stress or those with possible role in osmoregulation should be further characterized to reveal their involvement in controlling osmolarity in shrimp.