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Identification, cloning and expression analysis of Catechol-*O*-methyltransferase (COMT) gene from shrimp, *Penaeus monodon* and its relevance to salinity stress

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ABSTRACT

O-methyltransferase (OMT), a protein present ubiquitously in wide range of organisms plays significant role in methylation of small macro molecules for various functional and regulatory purposes. In crustaceans, OMT has functional role in growth, reproduction, ovarian development and molting. In the present study, suppression subtractive hybridization (SSH) performed using gill tissues of low (3ppt) and high (55ppt) salinity stressed shrimp *Penaeus monodon* resulted in identification of differentially expressed genes involved in signal transduction pathways, metabolism, defense proteins, DNA repair and synthesis, apoptosis, cell cycle regulation along with unknown and hypothetical proteins. Catechol-*O*-methyltransferase (COMT) a type of OMT was identified by SSH as one of the differentially expressed genes of shrimp *P. monodon* subjected to low and high salinity stress. The full length cDNA of COMT was cloned from the gills of *P. monodon* which consisted an open reading frame of 666 bp, encoding 221 amino acids. The ORF revealed one each of N-glycosylation and O-glycosylation sites and nine phosphorylation sites. The deduced amino acid sequence of COMT exhibited high sequence identity (92%) with COMT class of protein from *Fenneropenaeus chinensis*. Real time PCR analysis of the shrimp samples exposed to low salinity conditions at 3ppt revealed significant increase in expression of COMT transcripts in the guts at 24 h, 48 h, 1 week and 2 weeks, gills at 24 h and in the muscle tissues at 48 h, with maximum expression of the COMT levels by 5 fold in guts (1 week), 1 fold in gills (24 h) and 1.5 fold in muscle (48 h) respectively. The increased expression level of COMT at different time intervals in different tissues suggests a possible role of this gene in salinity stress tolerance in shrimps under low salinity conditions.

1. Introduction

O-methyltransferase (OMT) is an enzyme that is generally present across taxonomically diverse organisms which includes microbes [1], fungi [2], plants [3] and animals [4]. It catalyzes the methylation of both small and macro molecules for various functional and regulatory purposes. *O*-methylation in plants helps in variety of functions such as lignin biosynthesis, stress tolerance, and disease resistance [3]. Unlike in plants where OMTs are well characterized, the role of OMTs in microbes are not well understood [1,5] however, in general a variety of bacterial and fungal methyl transferases are reported to be involved in the biosynthesis of antibiotic [6] and aflatoxins [2] in addition to methylation of other compounds.

In crustaceans, farnesoic acid *O*-methyltransferase (FAMeT) has been reported to catalyze the methylation of farnesoic acid (FA) to produce isoprenoid methyl farnesoate (MF) which may regulate crustacean growth, reproduction, ovarian development and molting [7]. In shrimp *Metapenaeus ensis* constitutive FAMeT expression in multiple tissues with highest expression levels in the central nervous system has been reported [8]. The widespread cellular localization and endogenous activity of the FAMeT suggests that it might possess a wide spectrum of actions in different tissues that contribute to the function and regulation of MF synthesis in shrimp [9]. The role of FAMeT isolated from *Litopenaeus vannamei* was demonstrated to be involved in molting using RNA interference technique [10].

Catechol-*O*-methyltransferase (COMT) is another type of *O*-methyltransferases which catalyze the formation of methoxylated products by transferring methyl group from S-adenosyl-L-methionine to the hydroxyl group of catechol compounds. In shrimps COMT gene has been isolated and characterized from Chinese white shrimp *Fenneropenaeus chinensis* [7,11]. COMT transcripts were found to be constitutively expressed in haemocytes and tissues such as heart, hepatopancreas, stomach, gill, intestine and ovary with high expression levels in hepatopancreas and stomach of infected shrimp [7].

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In an attempt to identify the genes that play critical role in salinity tolerance, suppression subtractive hybridization (SSH) libraries were constructed from *P. monodon* gill tissues. In the present study, COMT gene was identified as one of the differentially expressed gene of shrimp subjected to salinity stress. This gene was cloned and characterized for the first time from shrimp *P. monodon* and the transcription profiles in various tissues during low (3ppt) and high (55ppt) salinity induced stress has been analyzed and discussed.

2. Materials and methods

2.1. Shrimp and tissue samples

P. monodon shrimps (10–15 g) were procured from the shrimp farms located in Chennai, India. The shrimps (intermolt stage) used in the experiment were divided into three groups of six numbers each and were acclimatized to three different salinity conditions. Low salinity levels (3ppt) were achieved by reducing the salinity of the sea-water by 2ppt per day by adding fresh water. High salinity levels (55ppt) were achieved by increasing the salinity by 2ppt per day using brine. The third group of shrimps was maintained in the normal sea-water (28ppt) as the control group, without altering the salinity conditions.

The gills, guts and muscles tissue samples from six shrimps maintained for a period of two weeks at low (3ppt), high (55ppt), and 28ppt salinity conditions were collected at different time intervals in RNA *later* solution (Qiagen, USA) and stored in −80 °C until further use.

2.2. Construction of SSH cDNA library

Six pooled gill tissues of low, high salinity and control group acclimatized shrimp samples were used for total RNA extraction, using NucleoSpin RNA II kit (Macherey–Nagel, Germany). The cDNA was synthesized, following the manufacturer's instructions, using a Super SMART-PCR cDNA Synthesis Kit (Clontech, USA). The SSH cDNA libraries for the low and high salinity induced gill tissue samples were constructed separately following the procedure described in PCR-Select cDNA subtraction kit (Clontech, USA). The "tester" constituted the low or high salinity exposed shrimp group cDNA whereas, the "driver" constituted the cDNA extracted from shrimp samples which were maintained in the normal sea water as the control group.

2.3. Screening of SSH cDNA library

The PCR products obtained by PCR amplification of SSH clones were ligated into pGEM-T Easy vector (Promega, USA) and transformed into competent *Escherichia coli* DH5α cells. The transformed *E. coli* cells were grown overnight onto LB agar plates with ampicillin (50 μg/ml). The recombinant clones were screened by colony PCR for the insert cDNAs with the vector primers (T7 forward and SP6) and sequenced (Scigenom technologies, India). Sequence analysis by BLASTX (www.ncbi.nlm.nih.gov/blast), BLASTN or TBLASTX were used to identify differentially expressed genes in cDNA libraries constructed from low and high salinity shrimp groups. The sequences were also analyzed using KEGG (Kyoto Encyclopedia of Genes and Genomes) software (www.genome.jp/kegg) and the sequences were grouped into contigs and singletons using 96 minimum percent identity for an alignment and overlap percent identity with cut off $N > 80$.

2.4. Amplification and cloning of P. monodon COMT gene

The COMT gene amplification of *P. monodon* was carried out using gene specific forward primer 5′-ATGTCTTCTCTGAAGAGTTA-3′ and reverse primer 5′-TCATTTTTTAAAACAGAGAGAC-3′. The primers were designed based on the reported open reading frame of *F. chinensis* COMT gene [7]. The PCR reaction conditions included initial denaturation at 94 °C for 2 min followed by 35 cycles of 94 °C for 30 s, 57 °C for 45 s, 72 °C for 1 min and final extension at 72 °C for 10 min. The PCR product of COMT gene was gel purified using PCR purification kit (Qiagen, USA) and cloned into pGEM-T Easy vector (Promega, USA). The cloned COMT gene was confirmed by sequencing.

2.5. Sequence analysis of P.monodon COMT gene

Nucleotide sequence analysis of *P. monodon* COMT was performed by BLASTN (http://www.ncbi.nlm.nih.gov/blast) [12] and the sequence was submitted in the GenBank database. COMT sequence was translated using the TRANSLATE tool in Expasy proteomics server (www.expasy.org). The multiple sequence alignment of COMT protein was performed by CLUSTALW method [13] in Expasy proteomics server (http://www.ebi.ac.uk/tools/msa/clustalw2/). Phylogenetic tree was constructed (PAM250) using MEGA 4.1 software [14] with the Neighbour-Joining method and bootstrap analysis was performed for 1000 replicates. The GenBank accession numbers of OMT related sequences from penaeids and other taxa that were analyzed include *Fenneropenaeus merguiensis long isoform* (DQ187995), *F. merguiensis* short isoform (DQ187994), *P. monodon* long isoform (DQ187993), *P. monodon* short isoform (DQ187992), *L. vannamei* isoform 2 (DQ067632), *L. vannamei* isoform 1 (DQ067631), *F. chinensis* (DQ091255), *Marsupenaeus japonicus* (AB247563) and *M. ensis* (AF333042), *Scylla paramamosain* (HQ587050), *Homo sapiens* (NM000754), *Ixodes scapularis* (XM002406515), *Arabidopsis thaliana* (NM105469), *Monodelphis domestica* (XM001366794), *Taeniopygia guttata* (XM002192637) and *Meleagris gallopavo* (XM003207958).

2.6. P. monodon COMT mRNA expression analysis by Real-time PCR

Total RNA extracted using NucleoSpin RNA II kit (Macherey-Nagel, Germany) from the gills, guts and muscle tissues of the control, low and high salinity stressed group shrimps was converted to cDNA with cDNA synthesis kit (Protoscript cDNA synthesis kit, New England Biolabs, USA). The cDNA was used to identify relative expression of COMT transcripts by real-time PCR using Power SYBR Green PCR master mix (Applied Biosystems, UK). The primers were designed with the aid of Primer Express software (Applied Biosystems, UK). The following primers for COMT (F:5′-CGTAATGCTGATCCTTTGGT-3′ and R:5′-CTCTACGGTGCTGC AGAGTT-3′) were used to generate 99 bp PCR product for real time analysis. The shrimp β-actin gene was amplified with the primers (F: 5′-GAACCTCTCGTTGCCGATGGTG-3′ and R: 5′-GAAGCTGTGC-TACGTGGCTCTG-3′) to generate 124 bp product which was used as an endogenous control. The relative quantification of the transcripts were assessed by comparative C_T method.

The real time PCR reaction was performed in triplicates in a final volume of 20 μl reaction mixture each. The reaction mixture contained cDNA (0.5 pmol), forward and reverse primer of β-actin or COMT gene (30.0 pmol) and 10 μl of 2X SYBR green master mix with the ROX dye (Applied Biosystems, UK). The reaction conditions for real time PCR was 95 °C for 1 min followed by 40 cycles of 95 °C for 15 s, 55 °C for 15 s and 72 °C for 30 s. The PCR amplification was followed by the melt curve conditions of 72 °C for 15 s, 55 °C for 10 s and 72 °C for 15 s. The relative quantification results were expressed as the fold change in levels of the gene expression and statistical analysis of the data for comparison between groups was carried out by one-way ANOVA and the values with $p < 0.05$ were considered significant.

3. Results

3.1. Identification and cloning of COMT gene

The SSH performed using gill tissues of salinity stressed shrimp *P. monodon* resulted in the identification of cDNA fragment as putative COMT. An approximate of 200 and 180 colonies were obtained in SSH cDNA library constructed from low and high salinity stressed shrimps respectively. The clones obtained from low salinity SSH libraries generated 15 contigs and 44 singletons and 8 contigs and 64 singletons from high salinity SSH library by KEGG analysis which could be represented under different functional category (Table 1 and Fig. 1A,B). The COMT gene was differentially regulated in both the SSH libraries generated from low salinity (3 times) and high salinity (2 times) experiments. The full length COMT gene amplification resulted in expected PCR product of 666 bp size. The ORF coding for protein with 221 amino acids revealed MW and pI of 24.2 kDa and 5.24 respectively (http://ca.expasy.org/tools/Compute pI/Mw). The complete ORF sequence of the COMT from *P.monodon* (Fig. 2) was submitted in the Genbank with the accession number (JN572540).

3.2. Nucleotide and amino acid sequence analysis

BLAST analysis revealed that the putative COMT from *P.monodon* (GenBank accession number JN572540) shared 92% and 94% nucleotide and amino acid sequence homology respectively with the reported OMT sequence from *F. chinensis* (DQ091255) which belongs to the COMT class of protein. The deduced amino acid sequence of *P. monodon* COMT showed identity with the FAMeT class of proteins from *Penaeus japonicus* (35%), *M. ensis* (33%), *P. merguiensis* (36%) and *L.vannamei* (36%).

The putative *P. monodon* COMT protein sequence analysis predicted one each of N-glycosylation and O-glycosylation sites (http://www.cbs.dtu.dk/services) and nine phosphorylation sites as analyzed by NetPhos 2.0 (www.cbs.dtu.dk/services/NetPhos). The protein displayed no signal peptide cleavage site as predicted by SignalP prediction (http://www.cbs.dtu.dk/services/SignalP). Protein analysis for identification of conserved domains indicated that the putative COMT consist of a 204 amino acid conserved domain from amino acid position 18–221 which belongs to methyl transferase-3-family that includes Catechol *O*-methyltransferase (COMT), caffeoyl-CoA *O*-methyltransferase (CCoAOMT) and a family of bacterial O-methyltransferases.

A total of 22 amino acid substitutions were identified between the *P. monodon* COMT and OMT from *F.chinensis* (Fig. 2). The phylogenetic analysis demonstrated the presence of putative COMT from *P. monodon* and *F. chinensis* in same clade (Fig. 3) and this class of COMT proteins could be differentiated from the other reported shrimp FAMeT class of proteins which were represented as a different cluster. Hence, the *P.monodon* COMT belongs to the S-Adenosyl methionine methyl transferase family of proteins similar to that of other methyl transferase group of proteins. This family includes COMT, CCoAOMT family of Indolethylamine N-methyltransferase from human and OMT from *Bacillus subtilis* [15]. NCBI conserved domain search of the *P. monodon* COMT revealed the presence of conserved domains possessed by superfamily of S-adenosylmethionine-dependent methyltransferases.

3.3. Quantification of P. monodon COMT transcripts in the tissues by real-time PCR

Real time PCR analysis of the shrimp samples exposed to low salinity conditions at 3ppt revealed significant increase in expression of COMT in the guts at 24 h, 48 h, 1 week and 2 weeks, gills at 24 h and in the muscle tissues at 48 h. No significant increase in expression levels of COMT in the shrimp samples exposed to high salinity conditions at 55ppt were observed when compared to the shrimp maintained as control group. The results of real time PCR carried out in shrimp tissues such as gills, guts and muscles are shown in Fig. 4.

Table 1
The differentially regulated genes identified in gills of low (3ppt) and high (55ppt) salinity acclimatized *P. monodon* shrimps.

Putative function	Acc. No	E. Value	Size (bp)	No of clones
Low salinity				
Defense related genes				
Catechol-*O*-methyltransferase	DQ091255	4e-75	222	3
Crustin-like antimicrobial peptide	ACL51682	3e-12	631	1
Metabolism				
Isocitrate dehydrogenase	ABX57390	1e-49	372	1
Cadherin	XP002578657	0.75	360	1
Cytochrome P450	YP001849240	9.5	158	1
Synthesis				
Ribonucleoside-diphosphate reductase subunit beta	YP001124919	6.4	559	1
RNA-dependent RNA polymerase homolog	AAB25907	0.001	560	1
ATP synthase subunit beta	XP003627732	1e-23	374	3
Others				
Hypothetical proteins				20
Putative transposase	EGR04439	6.9	335	1
Extracellular serine–threonine rich protein	GAA84625	9.2	279	1
Vacuolar protein sorting-associated protein	GAA82914	7.6	259	1
Collagen alpha-1(X) chain like	XP003204313	0.68	301	1
Unknown				23
High salinity				
Defense related genes				
Lysozyme	AAN86085	1e-30	578	1
Ferritin light chain-like	AAV84217	1e-04	330	1
Catechol-*O*-methyltransferase	DQ091255	0.0	570	2
DNA replication & repair				
Endonuclease-reverse transcriptase	ADI61818	0.002	387	1
Type II DNA modification enzyme	ZP04580500	4.5	864	1
Metabolism				
Phosphatidylinositol 4-kinase	XP003073890	0.73	242	1
FAD dependent oxidoreductase	EFY91406	4.4	112	1
Sterol desaturase	XP002516756	7.5	723	1
Membrane transporters and Signal transduction				
Fusion-associated small transmembrane protein	ACN38055	2.0	222	1
K^+-transporting ATPase, Beta subunit	EGQ63973	3.4	299	1
Ectodysplasin-A	EHB15808	0.35	312	1
ATP-binding cassette A	EAW73058	2.4	128	1
Plasmodium membrane protein	XP001347495	1.3	200	1
Apoptosis and cell cycle regulators				
Programmed cell death 6-interacting protein	XP003385263	0.18	165	1
Testis-expressed sequence 10 protein-like protein	EGI70386	7.8	149	1
Others				
Hypothetical proteins				20
Unknown				28

4. Discussion

Penaeid shrimps in general can survive in a wide range of salinities (3–50ppt) and are cultured under a variety of conditions in many tropical and subtropical areas of the world. However, abiotic and biotic stress factors may result in stress to the animals during culture period. The cultured shrimps generally are exposed to climatic changes that affect the physico-chemical quality of

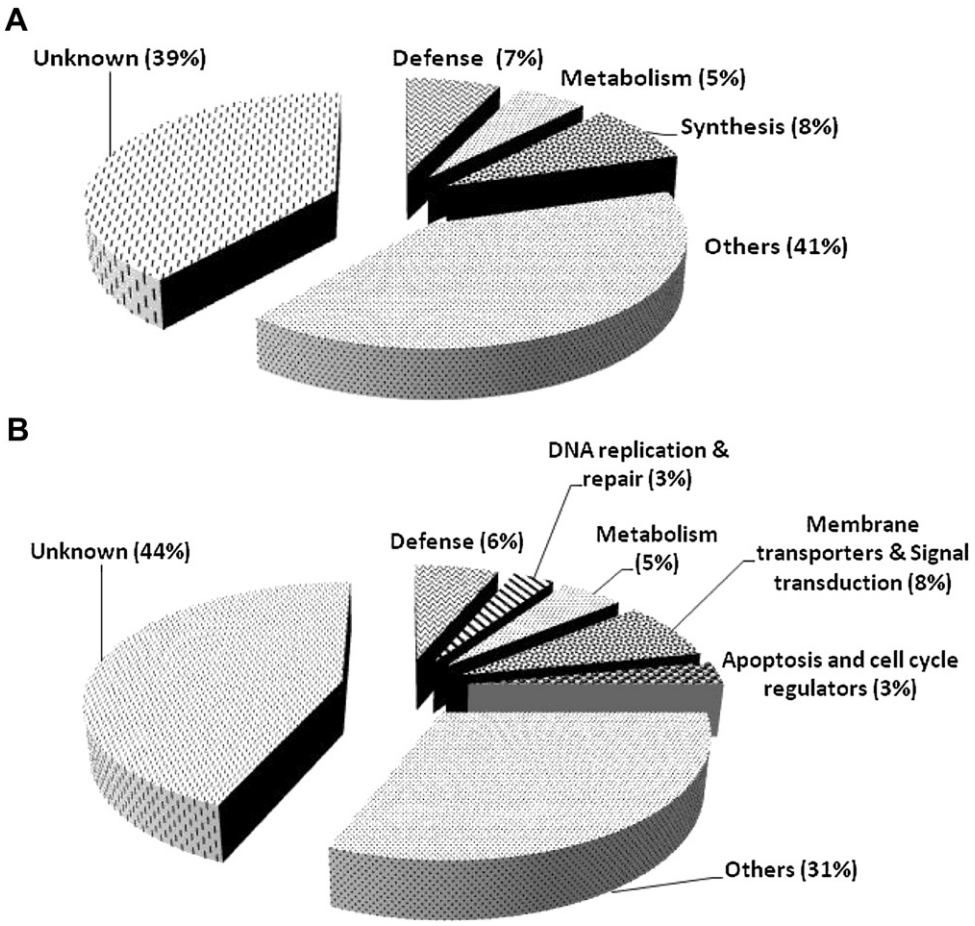

Fig. 1. A. Differentially expressed genes from gill tissues of *P. monodon* shrimps under low salinity conditions by SSH library construction. B. Differentially expressed genes from gill tissues of *P. monodon* shrimps under high salinity conditions by SSH library construction.

water. Among these changes of the seawater physico-chemical characteristics, the salinity and temperature have great impact on euryhaline penaeids, affecting their metabolism, growth, molting and survival [16,17]. Drastic salinity changes may also affect the feed intake and higher energy utilization for osmoregulation resulting in poor growth. In shrimps, the biochemical and physiological response to salinity changes are widely reported [18,19] however, molecular studies on differential expression of genes and their role in salinity tolerance remains poor.

Sequence analysis of the COMT from *P. monodon* in the present study, revealed high sequence similarity with the reported COMT from *F. chinensis* and the results from the phylogenetic tree analysis indicated that the OMT from *P. monodon* and *F. chinensis* belongs to COMT class of proteins which could be differentiated from the FAMeT class of proteins which was represented in a different cluster. Therefore, the OMT of *P. monodon* shrimp isolated in the present study appears to belong to the COMT group and hence, may be considered as a member of the COMT group. The COMT protein sequence revealed no signal peptide cleavage site as analyzed by SignalP. Similar observation of lack of signal peptide has been reported for the putative *F. chinensis* COMT [7]. The absence of signal peptide has also been reported in *L. vannamei* FAMeT, suggesting that these are expressed as cytosolic protein that remain in the cytoplasm and is not secreted into the circulation [10].

The *P. monodon* COMT sequence analysis revealed the presence of putative multiple phosphorylation sites. Similar putative multiple phosphorylation sites has been reported for COMT of *F. chinensis* [7]. The *P. monodon* COMT posses conserved domains similar to that present in the members of the *O*-methyltransferases, which includes COMT, CCoAOMT and a family of bacterial *O*-methyltransferases that may be involved in antibiotic production. A total of 22 amino acid substitutions which were identified between the *P. monodon* COMT and OMT from *F.chinensis* sequences in the present study may differ as the primers to amplify the ORF of *P. monodon* COMT were based on *F. chinensis* reported sequence.

The multiple isoforms of FAMeT's isolated from crustaceans [20] have been characterized to some extent with respect to its function such as involvement in methylfarnesoate biosynthetic pathway [21], molting [10] and tissue distribution [9], whereas, the role of COMT in humans has been reported to be involved in the inactivation of catechol estrogens [22]. The COMT helps in detoxification through methylation of catechol estrogens inhibiting the redox cycling, thereby decreasing reactive oxygen species formation [23]. However, COMT in shrimps need further characterization at molecular and biochemical levels to decipher the functional role of this enzyme.

Osmotic and ionic regulation is an important mechanism of environmental adaptation in crustaceans and tissues such as gills and guts are the primary osmoregulatory organs in penaeids [24]. Since molting in shrimps involve physiological changes in the muscles [25], the expression of COMT transcripts in muscle was assessed. Hence, the relative expression of COMT was analyzed in gills, guts and muscle tissues of *P. monodon*. The *P. monodon* COMT was found to be upregulated in low salinity stress conditions at different time intervals in shrimp tissues (gills, guts and muscles). Relative expression analysis suggests that the significant increase in

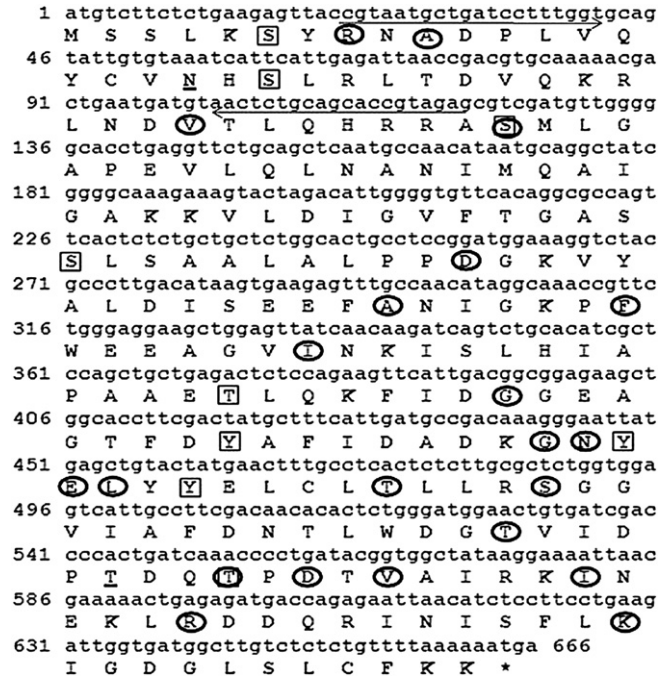

Fig. 2. The nucleotide and deduced amino acid sequence of putative *P. monodon* COMT The nine potential phosphorylation sites are boxed and each of the N-glycosylation and O-glycosylation sites are underlined. The arrows represent primers designed for real time PCR analysis. The circles represent amino acids in *P. monodon* COMT which differ from the OMT sequence of *F. chinensis*.

expression of COMT was induced at 24 h in gut and gills and at 48 h in the muscle tissue of *P. monodon* under low salinity stress (3ppt). The COMT expression was maximum at 1-week time interval by 5 folds in the gut tissues suggesting a significant function of COMT in this tissue. The COMT gene expression appears to be enhanced under high (55ppt) salinity conditions in gills (2 weeks), gut (2 weeks) and muscles (24 h) however, we observed statistically insignificant changes in the expression levels of COMT under high salinity conditions unlike in shrimps which were exposed to low salinity conditions. This difference in expression of COMT may be due to exposure of shrimp under such a wide range of salinity conditions. The shrimps must adapt physiologically to counteract with osmotic pressure and ionic strength of the rearing environment. Adaption of osmoregulatory organs to different salinity environments with enzymatic activity changes has been reported in crustaceans. For example, at low salinity conditions (7ppt), drastic subcellular changes have been observed in antennal glands of shrimps (*P. monodon*). The ultrastructural changes were associated with alterations in the expression and activity of Na^+/K^+-ATPase in shrimps subjected to various salinities [26]. In crustaceans, the antennal gland is known to regulate osmolarity in the hemolymph and body fluid through sodium and potassium fluxes by means of Na^+/K^+-ATPase activity [27]. The Na^+/K^+-ATPase activity in the antennal gland of mangrove crab, *Ucides cordatus*, reared under 9ppt was found higher than that of crabs maintained under 26 ppt [28].

With respect to another important enzyme carbonic anhydrase (CA), which is involved in ion uptake in shrimps, several studies have shown that the expression of CA mRNA and activity is elevated in a low salinity environment. For example, in euryhaline crab at 10 ppt salinity, the expression and activity of CA protein in crab gills were shown to be up-regulated by 5–10 fold [29–31]. In white

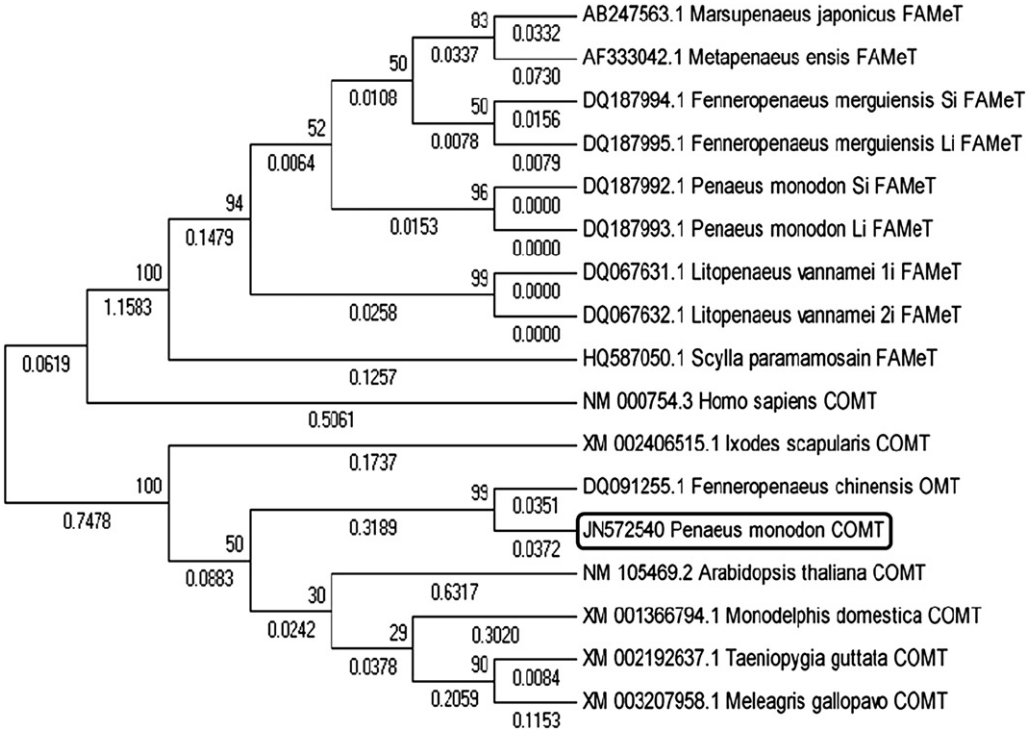

Fig. 3. Phylogenetic analysis of *P. monodon* Catechol *O*-methyltransferase protein (JN572540) with *Fenneropenaeus merguisis* long isoform (DQ187995), *F. merguiensis* short isoform (DQ187994), *P. monodon* isoforms (DQ187993 and DQ187992), *Litopenaeus vannamei* isoforms (DQ067632 and DQ067631), *F. chinensis* (DQ091255) and *Marsupenaeus japonicus* (AB247563), *Scylla paramamosain* (HQ587050), *Homo sapiens* (NM000754), *Ixodes scapularis* (XM002406515), *Arabidopsis thaliana* (NM105469), *Monodelphis domestica* (XM001366794), *Taeniopygia guttata* (XM002192637), and *Meleagris gallopavo* (XM003207958). The *P. monodon* Catechol *O*-methyltransferase is indicated in box.

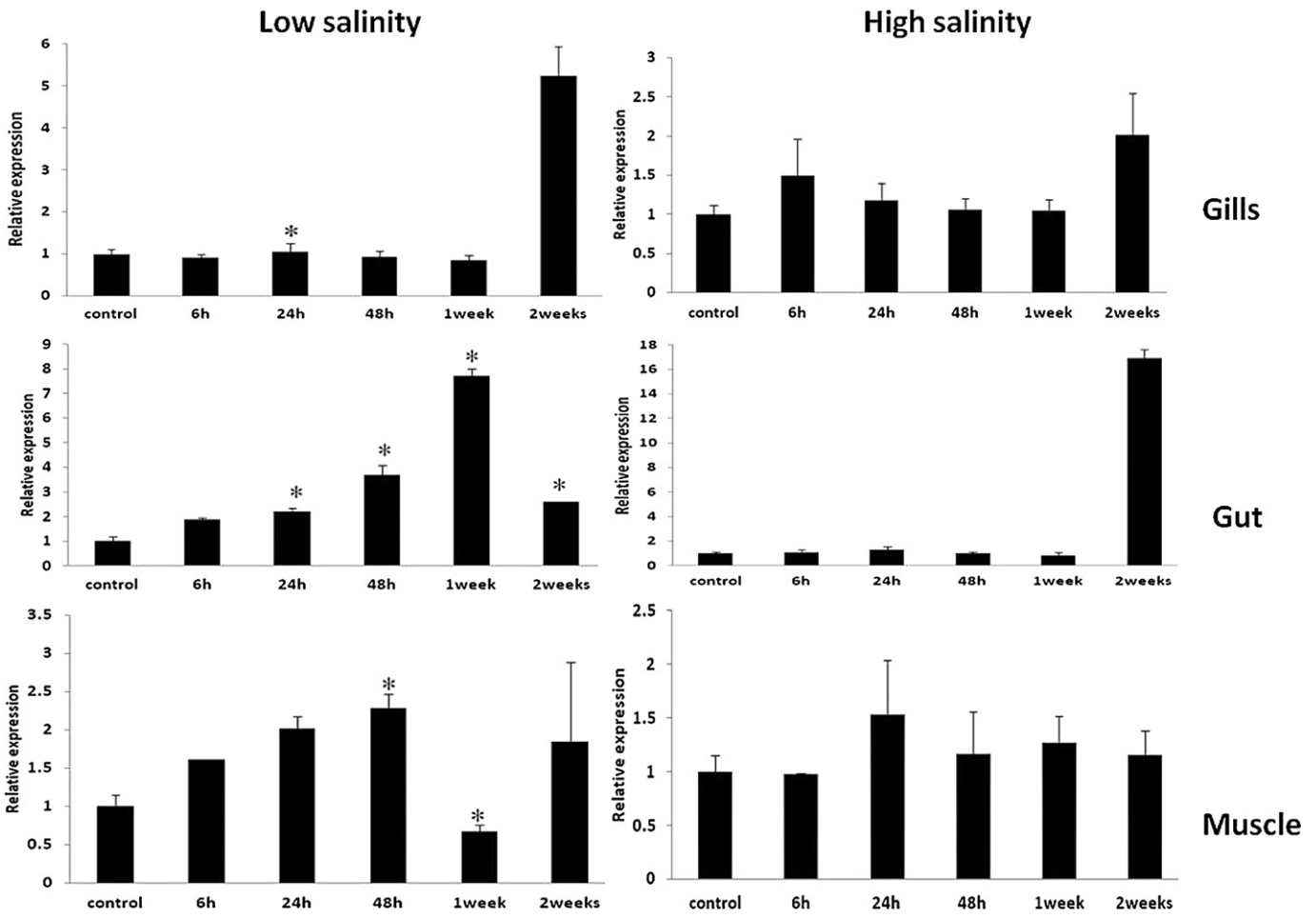

Fig. 4. Analysis of *P. monodon* Catechol *O*-methyltransferase transcripts expression in tissues (gills, guts and muscle) under low and high salinity stress by real-time PCR at 6 h, 24 h, 48 h, 1 week and 2 weeks time intervals. The significant difference ($p < 0.05$) in OMT expression levels are indicated with asterisks.

shrimp *L. vannamei*, the CA activity was reported to be up-regulated in gills tissues of shrimps exposed to low salinity at 5 and 15ppt by approximately 1.5–4 folds [32] and in the gills of the black tiger shrimp approximately 1.3–1.4 folds increased induction was observed when the shrimps were transferred from 25ppt to 3ppt salinity [33].

The identification of COMT gene however, in both low and high salinity SSH libraries suggest that the protein might have a crucial role during salinity induced stress in the shrimp. Differential expression of OMT genes by SSH has been reported in Atlantic salmon during infection [34]. These studies indicate that OMT may have significant role in regulation of immune function in addition to stress in shrimps which needs to be further investigated. The widespread tissue expression pattern of FAMeTs in crustaceans such as *M. ensis* [8] and *Cancer pagarus* [35], the ubiquitous distribution of COMT in tissues of *F. chinensis* [7] and COMT expression in gut, gills and muscle tissues of *P. monodon* in this study indicates that these enzymes may possess multiple functions in the shrimp which remains to be characterized at molecular level.

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References

[1] Yoon Y, Park Y, Yi YS, Lee Y, Jo G, Park JC, et al. Characterization of an O-methyltransferase from *Streptomyces avermitilis*. MA-4680. J Microbiol Biotechnol 2010;20:1359–66.
[2] Yu J, Cary JW, Bhatnagar D, Cleveland TE, Keller NP, Chu FS. Cloning and characterization of a cDNA from *Aspergillus parasiticus* encoding an O-methyltransferase involved in aflatoxin biosynthesis. Appl Environ Microbiol 1993; 59:3564–71.
[3] Lam KC, Ibrahim RK, Behdad B, Dayanandan S. Structure, function, and evolution of plant O-methyltransferases. Genome 2007;50:1001–13.
[4] Bonifacio MJ, Vieira-Coelho MA, Borges N, Soares-da-Silva P. Kinetics of rat brain and liver solubilized membrane-bound catechol-O methyltransferase. Arch Biochem Biophys 2000;384:361–7.
[5] Kristina AEL, Zetterlunda I, Delp G, Lisbeth MVJ. N-Methyltransferase involved in gramine biosynthesis in barley: cloning and characterization. Phytochemistry 2006;67:2002–8.
[6] Connors NC, Strohl WR. Partial purification and properties of carminomycin 4-O-methyltransferase from *Streptomyces* sp. strain C5. J Gen Microbiol 1993; 139:1353–62.
[7] Li DX, Du XJ, Zhao XF, Wang JX. Cloning and expression analysis of an O-Methyltransferase (OMT) gene from Chinese shrimp, *Fenneropenaeus chinensis*. Fish Shellfish Immunol 2006;21:284–92.
[8] Gunawardene YI, Tobe SS, Bendena WG, Chow BK, Yagi KJ, Chan SM. Function and cellular localization of farnesoic acid O-methyltransferase (FAMeT) in the shrimp, *Metapenaeus ensis*. Eur. J Biochem 2002;269:3587–95.
[9] Gunawardene YI, Bendena WG, Tobe SS, Chan SM. Comparative immuno histochemistry and cellular distribution of farnesoic acid O-methyl transferase in the shrimp and the crayfish. Peptides 2003;24:1591–7.
[10] Hui JHL, Tobe SS, Chan SM. Characterization of the putative farnesoic acid O-methyltransferase (LvFAMeT) cDNA from white shrimp, *Litopenaeus vannamei*: evidence for its role in molting. Peptides 2008;29:252–60.
[11] Li DX, Du XJ, Zhao XF, Expression Wang JX. Purification and activity assay of the recombinant protein of Catechol-O-methyl transferase from Chinese

white shrimp (*Fenneropenaeus chinensis*). Am J Biochem Biotechnol 2010;6: 148–54.
[12] Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 1997;25:3389–402.
[13] Higgins DG, Bleasby AJ. R. Clustalv improved software for multiple sequence alignment. Comput Appl Biosci 1992;8:189–91.
[14] Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 2011;28: 2731–9.
[15] Kodama KI, Nakabeppu Y, Sekiguchi M. Cloning and expression of the *Bacillus subtilis* methyltransferase gene in *Escherichia coli* ada- cells. Mutat Res 1989; 218:153–63.
[16] Staples DJ, Heales DS. Temperature and salinity optima for growth and survival of juvenile banana prawns *Penaeus merguiensis*. J Exp Mar Biol Ecol 1991;154:251–74.
[17] Chen JC, Lin MN, Ting YY, Lin JN. Survival, haemolymph osmolality and tissue water of *Penaeus chinensis* juveniles acclimated to different salinities and temperature levels. Comp Biochem Physiol A 1995;110:253–8.
[18] Chen JC, Lin JL. Osmolality and chloride concentration in the hemolymph of subadult *Penaeus chinensis* subjected to different salinity level. Aquaculture 1994;125:167–74.
[19] Perazzolo LM, Gargioni R, Ogliari P, Barracco MAA. Evaluation of some hemato-immunological parameters in the shrimp *Farfantepenaeus paulensis* submitted to environmental and physiological stress. Aquaculture 2002;214:19–33.
[20] Kuballa AV, Guyatt K, Dixon B, Thaggard H, Ashton AR, Paterson B, et al. Isolation and expression analysis of multiple isoforms of putative farnesoic acid *O*-methyltransferase in several crustacean species. Gen Comp Endocrinol 2007;150:48–58.
[21] Gunawardene YI, Chow BK, He JG, Chan SM. The shrimp FAMeT cDNA is encoded for a putative enzyme involved in the methylfarnesoate (MF) biosynthetic pathway and is temporally expressed in the eyestalk of different sexes. Insect Biochem Mol Biol 2001;31:1115–24.
[22] Weisz J, Clawson GA, Creveling CR. Biogenesis and inactivation of catecholestrogens. Adv Pharmacol 1998;42:828–33.
[23] Matsui A, Ikeda T, Enomoto K, Hosoda K, Nakashima H, Omae K, et al. Increased formation of oxidative DNA damage, 8 hydroxy -2'- deoxy guanosine in human breast cancer tissue and its relationship to GSTP1 and COMT genotypes. Cancer Lett 2000;151:87–95.
[24] Péqueux A. Osmotic regulation in Crustaceans. J Crustac Biol 1995;15:1–60.
[25] Cesar JRO, Zhao B, Malecha S, Ako H, Yang J. Morphological and biochemical changes in the muscle of the marine shrimp *Litopenaeus vannamei* during the molt cycle. Aquaculture 2006;261:688–94.
[26] Buranajitpirom D, Asuvapongpatana S, Weerachatyanukul W, Wongprasert K, Namwong W, Poltana P, et al. Adaptation of the black tiger shrimp, *Penaeus monodon*, to different salinities through an excretory function of the antennal gland. Cell Tissue Res 2010;340:481–9.
[27] Cieluch U, Anger K, Aujoulat F, Buchholz F, Charmantier-Daures M, Charmantier G. Ontogeny of osmoregulatory structures and functions in the green crab *Carcinus maenas* (Crustacea, Decapoda). J Exp Biol 2004;207: 325–36.
[28] Harris RR, Santos MCF. Sodium uptake and transport ($Na^+ + K^+$)ATPase changes following Na^+ depletion and low salinity acclimation in the mangrove crab *Ucides cordatus* (L.). Comp Biochem Physiol 1993;105:35–42.
[29] Henry RP, Garrelts EE, McCarty MM, Towle DW. Differential induction of branchial carbonic anhydrase and Na^+/K^+ ATPase activity in the euryhaline crab, *Carcinus maenas*, in response to low salinity exposure. J Exp Zool 2002; 292:595–603.
[30] Henry RP, Gehnrich S, Weihrauch D, Towle DW. Salinity-mediated carbonic anhydrase induction in the gills of the euryhaline green crab, *Carcinus maenas*. Comp Biochem Physiol A 2003;136:243–58.
[31] Henry RP, Thomason KL, Towle DW. Quantitative changes in branchial carbonic anhydrase activity and expression in the euryhaline green crab, *Carcinus maenas*, in response to low salinity exposure. J Exp Zool 2006;305: 842–50.
[32] Roy LA, Davis DA, Saoud IP, Henry RP. Branchial carbonic anhydrase activity and ninhydrin positive substances in the Pacific white shrimp, *Litopenaeus vannamei*, acclimated to low and high salinities. Comp Biochem Physiol A 2007;147:404–11.
[33] Pongsomboon S, Udomlertpreecha S, Amparyup P, Wuthisuthimethavee S, Tassanakajon A. Gene expression and activity of carbonic anhydrase in salinity stressed *Penaeus monodon*. Comp Biochem Physiol A 2009;152:225–33.
[34] Tsoi SCM, Ewart KV, Penny S, Melville K, Liebscher RS, Brown LL, et al. Identification of immune-relevant genes from Atlantic salmon using suppression subtractive hybridization. Mar Biotechnol 2004;6:199–214.
[35] Ruddell CJ, Wainwright G, Geffen A, White MRH, Webster SG, Rees HH. Cloning, characterization and developmental expression of a putative farnesoic acid O-methyltransferase in the female edible crab *Cancer pagurus*. Biol Bull 2003;205:308–18.