CHAPTER V
CONCLUSIONS

1. Genes involved in osmoregulation were identified from *P. monodon* tissues; antennal gland, epipodite and gill, by DD-PCR technique. By comparing DD-PCR profiles of stressed shrimp (3 and 40 ppt salinities) and the control (25 ppt salinity), 97 differentially expressed bands were identified from seventeen combinations of primers. These bands were successfully reamplified, cloned and sequenced.

2. Sequence analysis revealed a total of 129 unique sequences. Twenty-two percentage of DD-PCR bands contained one sequence, whereas the remaining 78% of the bands were the mixture of different sequences arisen from co-migration of non-homologous cDNA or a technical error.

3. Homology search of 129 unique sequences found that 37 different sequences were significantly matched with the GenBank database entries, whereas 92 different sequences were not significantly matched. Among these sequences, 22, 6 and 9 unique sequences were matched to known genes, ribosomal proteins and hypothetical proteins, respectively.

4. Twenty-three of the 129 unique sequences originally identified from DD-PCR as potentially regulated by salinity stress were chosen to confirm their differential expression using semi-quantitative RT-PCR. The results confirmed the differential expression of 21 cDNA fragment under salinity stress. These 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. The relative expression of the remaining 2 cDNA fragments (S34 and S111) encoding for sarcolemmal associated protein 2 and

unknown gene, respectively, did not significantly alter in response to high or low salinity stress.

5. cDNA fragments encoding for unknown genes were predicted for their protein functions by performing domain prediction with SMART program and Kyte-Doolittle hydropathy plots. The predicted amino acid sequences of S5, S114 and S126 cDNA fragments had potential to be a transmembrane region.