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**THE EFFECT OF SALINITY EXPOSURE ON
THE GENE EXPRESSION IN THE
Pangasius nasutus (BLEEKER, 1863) MUSCLE**

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**Thesis Submitted in Fulfillment of the Requirement
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**THE EFFECT OF SALINITY EXPOSURE ON THE GENE EXPRESSION IN
THE *Pangasius nasutus* (BLEEKER, 1863) MUSCLE**

NABILAH BINTI MOHAMAD ALI

2013

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Faculty : Fisheries and Aqua-Industry

Salt has been the traditional remedy used in aquaculture industry which is also widely applied in the culture practice of pangasids. With the extensive use of salt in the aquaculture industry, the study on *Pangasius nasutus* molecular responses in the muscle tissue towards salinity exposure is still limited. Due to this, a study was carried out to identify genes that were expressed during salinity exposure in the muscle tissue of *P. nasutus*. A total of six juvenile fishes were placed in a separate 150 L aquarium tanks with different salinities (10 ppt, 15 ppt and 20 ppt) for two weeks and 0 ppt acts as control. The differential display reverse transcriptase PCR (DDRT-PCR) was used to identify the differentially expressed cDNA fragments between treatments. Real Time PCR was carried out to determine the regulation of selected genes that have been expressed in the muscle tissue. 20 ACP primers were used in amplification, the differential display profile showed that 18 cDNA bands were up-regulated in 20 ppt and two were found up-regulated in 10 ppt. The identification of the differentially expressed genes (DEGs) sequences using BLASTN showed 14 DEG were found to be homologous with nine known genes in the GenBank; fructose-biphosphate aldolase A (ALDOA), troponin I (TnI), myosin heavy chain (MYH), myosin light chain 1a (MLC 1a), creatine kinase (CK), *ATPase*

subunit 8 and 6 (*ATPase 8/6*), parvalbumin (PRV), ribosomal protein L26 (RPL26) and L11 (RPL11). The real time quantitative PCR analysis showed that different salinity exposure has triggered the differential expression of troponin I (TnI), myosin heavy chain (MYH), parvalbumin (PV) and growth hormone (GH) genes in the muscle tissue of *P. nasutus*. The expression of TnI, MYH and PV were highest in the 20 ppt salinity treatment with 8.79, 3.76 and 1.79 fold inductions over control (0 ppt) respectively. Meanwhile, the expression of GH gene was found highest in 10 ppt salinity treatment with 10.51 fold inductions over control (0 ppt). This result showed that salinity exposure can induce the expression of several genes in *P. nasutus* muscle tissue. However further study needs to be carried out in order to have better understanding of the gene function towards salinity exposure.

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**KESAN PENDEDAHAN SALINITI KE ATAS PENGEKSPRESAN GEN DI
DALAM OTOT *Pangasius nasutus* (BLEEKER, 1863)**

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Garam adalah rawatan tradisional yang telah digunakan di dalam industri akuakultur di mana ia juga telah digunakan secara meluas dalam pembiakan ikan pangasids. Dengan penggunaan yang meluas dalam industri akuakultur, kajian ke atas tindak balas molekular *Pangasius nasutus* terhadap pendedahan saliniti di dalam tisu otot masih lagi terhad. Kajian telah dilakukan untuk mengenal pasti beberapa gen yang telah diekspreskan di dalam tisu otot *P. nasutus* semasa pendedahan saliniti. Sejumlah enam ikan juvenil telah diletakkan di dalam akuarium 150 L yang berbeza dengan saliniti yang berlainan (10 ppt, 15 ppt, 20 ppt) untuk dua minggu dengan air 0 ppt sebagai kawalan. Kaedah “differential display reverse transcriptase” PCR (DDRT-PCR) telah digunakan untuk mengenal pasti fragmen cDNA yang telah diekspreskan secara berbeza di dalam setiap rawatan. Analisis Real Time PCR digunakan untuk mengenal pasti regulasi beberapa gen terpilih yang telah diekspreskan di dalam otot. 20 ACP primer telah digunakan semasa amplifikasi, profil differential display telah menunjukkan 18 fragmen cDNA diekspreskan secara berbeza dalam sampel 20 ppt dan dua dalam sampel 10 ppt. Identifikasi jujukan “differentially expressed genes” (DEGs) menggunakan BLASTN telah menunjukkan bahawa 14 DEG mempunyai persamaan dengan sembilan gen-gen di dalam

GenBank; fructose-biphosphate aldolase A (ALDOA), troponin I (TnI), myosin heavy chain (MYH), myosin light chain 1a (MLC 1a), creatine kinase (CK), ATPase subunit 8 and 6 (ATPase 8/6), parvalbumin (PRV), ribosomal protein L26 (RPL26) and L11 (RPL11). Analisis real time quantitative PCR menunjukkan pendedahan kepada saliniti berbeza telah mencetuskan perbezaan dalam jumlah pengekspresan gen seperti troponin I (TnI), myosin heavy chain (MYH), parvalbumin (PV) dan growth hormone (GH) di dalam tisu otot *P. nasutus*. Pengekspresan gen TnI, MYH dan PV adalah tertinggi di dalam rawatan 20 ppt dengan peningkatan fold sebanyak 8.79, 3.76 dan 1.79 ke atas kawalan (0 ppt). Manakala pengekspresan gen GH adalah tertinggi di dalam rawatan 10 ppt dengan peningkatan fold sebanyak 10.51 ke atas kawalan (0 ppt). Keputusan in telah menunjukkan yang pendedahan kepada salinity boleh mempengaruhi pengekspresan beberapa gen di dalam otot *P. nasutus*. Walau bagaimana pun, kajian lebih mendalam haruslah diteruskan untuk mengenal pasti kesan pendedahan saliniti ke atas fungsi-fungsi gen tersebut pada masa hadapan.