- Invited Paper -

IDENTIFICATION OF ECONOMICALLY IMPORTANT TRAITS IN FISH USING MICROARRAY

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Microarrays are a new technology that consist of hundreds to thousands of genes robotically arrayed on specially treated glass slides. By labeling two different RNA samples with different fluorescent dyes (Cy 3- and Cy 5-dCTP), microarrays can identify which genes are up or down regulated between the two conditions tested. The ability to survey the expression pattern of thousands of genes at once has major implications to the improvement of modern aquaculture species improvement. By comparing two closely related lines that differ in a specific traits using microarray technology it is possible to identify the genes that contribute to that trait. These genes can then be altered via a transgenic approach or selectively breed into elite lines.

Microarray analysis can also be combined with quantitative trait loci analysis to provide not only valuable molecular markers linked to the trait, but also the ability to understand the molecular basis of the trait by finding genes whose expression are closely associated with the phenotype under investigation. Thus, microarray technology provides a new way of understanding important complex traits (growth rate, dress out, feed conversion, reproduction, diseases resistance, and so on) that will provide the basis for developing more rational strategies in modifying aquaculture species architecture and physiology. The objective of this study is the development of a method for selective breeding of aquaculture species by using the cDNA microarray.

For microarray analysis, thousands of DNA sequence information will be necessary. Recently, advances in genetic analysis technology were remarkable especially in the determination of DNA sequencing. It brings possible the acquisition of huge quantities of genes information for a short period of time using the expressed sequence tags (EST) analysis. Previously, we analyzed and identified more than 2,500 ESTs of Japanese flounder liver, spleen , kidney, skin, and peripheral blood leukocytes (PBL) cDNAs. In this study, we used some of these cDNAs for microarray analysis using mRNA from in vitro stimulated kidney and PBL cells as the 1st step in the development of a method for selective breeding of aquaculture species.

The preparation of the DNA chip was performed as follows : Japanese flounder cDNA clones were made as candidates in order not to choose the same gene from our previous EST analysis. In order to amplify the cDNA region, PCR reaction was carried out under the

following condition. cDNA clone of EST was used as template DNA and primers were designed to anneal the vector region (sense 5' -GTGCTGCAAGGCGATTAAGTTGG-3' , antisense 5' -TCCGGCTCGTATGTTGTGTGGA-3'). The sample was preheated at 95℃ for 5 min and subjected to 40 cycles of amplification each consisting of 30 sec at 95℃, 30 sec at 55℃, 2 min at 72℃, with a 5 min extension at 72℃ during the last step. PCR products were purified and concentrated using 96 PCR cleanup kit and its final concentration was above 500 ㎍/ml. Spotting the purified PCR products to slide glass were processed by DNA Chip Research Inc. 871 clones were spotted on slide glass consisting of 500 known genes and 371 functionally unknown genes.

PBLs were stimulated with 0.1 ㎍/ml of PMA or LPS 0.5 mg/ml of LPS and samples were taken at 0, 1 and 3 hours after stimulation. Kidney cells were stimulated with 0.05 ㎍ /ml of Con A, 0.1 ㎍/ml of PMA, 0.5 mg/ml of LPS, and 10 $TCID_{50}$ of Hirame rhabdo virus (HRV). Samples were taken at 0, 1, 3 and 6 hrs after stimulation. Total RNA was isolated from PBLs and kidney cells and then mRNA was purified from total RNA. 1 ㎍ of purified mRNA from controls and stimulated samples were labeled respectively with Cy3 and Cy5-dCTP. The arrayed clones were hybridized with equal volume of Cy3-or Cy5-labeled first strand cDNA in hybridization buffer. Hybridization was carried out for 16 hrs at 42℃. After 16 hrs, the slide glasses were washed with 2x SSC-0.1 % SDS for 20 min at room temperature, 0.2x SSC-0.1 % SDS for 20 min at room temperature, two washes 0.2x SSC-0.1 % SDS for 20 min at 55℃ with shaking, followed by one rinse of 0.2x SSC-0.1 % SDS at room temperature. The micoarrays were dried and scanned immediately using a GenePix 4000B. Images obtained from scanning were analyzed by manufactured software (GenePix Pro ver. 3.0). Normalization was performed to equibrate Cy3 and Cy5 signal intensity with the spotted housekeeping gene, β-actin. The signal intensity was calculated from duplicate spotted clone intensity means and subtracted the background signal. Feature ratio over 2.0 was considered as up-regulated gene and lower than 0.5 was considered as down-regulated gene.

To investigate the gene expression pattern during the early stages of viral infection and cell homeostasis related genes under carcinogenesis induction treatment, kidney cell or PBL were infected with HRV or treated with Con A, PMA or LPS. The up- or down-regulated gene number by Con A is 6 (0.7%) and 11 (1.3%) at 1 hour and 6 hours stimulation, respectively. By PMA stimulation, 62 (7.4%) and 77 (9.3%) of the gene expression level was changed at 1 hour and 6 hours stimulation, respectively at both 1 hour and 6 hours stimulation by PMA, more than 50 gene expression levels were repressed. By LPS stimulation, 25 (3%) and 51 (6.1%) gene expression levels were changed at 1 hour and 6 hours stimulation, respectively. Especially, 49 genes were induced by LPS at 6 hour stimulation and a few genes were repressed. With HRV infection, 148 (18%) and 34 (4.1%) gene expression levels were changed at 3 hour and 6 hours post infection, respectively. 139 gene expressions were induced after 3 hours infection but by 6 hours. PBLs were stimulated by LPS for 1 hour and 3 hours, from which 34 (4.1%) and 73 (8.8%) gene

expression levels were changed, respectively. By PMA stimulation, 32 (3.8 %) gene expression levels were changed.

From the gene expression profile, we could get lots of information about the dynamic change in the gene expression of the cells under different stress or stimulations. Microarray technology made possible the analysis of huge number of clones rapidly. All of our studies suggest that the microarray analysis is a powerful method to characterize varying gene expression in different types of cells at different stress factors, and has an application in the detection of different phenotypic characteristics of organisms at the gene expression level. It is expected that further microarray analysis must help in the development of a method for selective breeding of aquaculture species.