EST projects in aquaculture: sea bass, red tuna and perch

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RIAASUNTO – Progetto EST in acquacoltura: spigola, tonno rosso e persico. Queste tre specie stanno richiamando un sempre maggior interesse. Si è reso quindi necessario effettuare un programma che permetta di valutare le abitudini comportamentali dei pesci di allevamento e gli eventuali problemi nelle varie fasi del ciclo biologico indagando, per esempio, l’espressione di geni coinvolti nella maturazione dei gameti e nella fecondazione e/o quelli che hanno un ruolo nella risposta di tipo biologico agli stressori ambientali. A tal fine abbiamo dato il via a questo progetto preparando una library di cDNA di fegato di branzino e sequenziando alcune centinaia di cloni il 55% dei quali risultano essere sequenze singole, mentre i restanti vanno a formare dei contig. Contemporaneamente è in corso uno studio sulle gonadi di tonno rosso e sul fegato di persico. L’obiettivo di questo progetto è quello di creare database di sequenze di cDNA di queste tre specie di pesci ad interesse commerciale. Di tali library verranno sequenziati circa 5.000 cloni; le sequenze ottenute, depositate in banca dati, saranno disponibili via Internet a tutta la comunità scientifica che le potrà utilizzare per le ricerche più disparate.

Key words: welfare, cDNA library, molecular biology.

INTRODUCTION – The modern technologies used in aquaculture may improve fish production and quality and, at the same time, reduce environmental impact with benefits on the public perception of the industry. To be economically profitable, these modern technologies request an increase of rearing density that, however, could affect fish welfare (Vazzana et al., 2002). Therefore, beside the traditional markers, it may be important to look for alternative parameters such as molecular biomarkers (Gornati et al., 2004; Ryan and Hightower, 1996). In this view, genomic strategies are revolutionizing scientific research also in the understanding of fish physiology and gene evolution; this is also due to the relatively easiness to isolate novel and homologous genes using public databases (Parrington and Covard, 2002).

The crucial point in the every-day management of fish farming, using molecular biomarkers, is not inside the methods, but is due to the scarcity of the genomic resources for some commercial fish species, in spite of their economical interest. For this reason we have started an EST project on sea bass, red tuna and perch. In this paper we present the preliminary results on some EST sequence obtained from a liver sea bass cDNA library.

MATERIALS AND METHODS – cDNA library construction: we have constructed a cDNA library from normal liver of D. labrax using ZAP-cDNA Gigapack III Gold Cloning Kit (Stratagene) following the manufacturered instruction. Briefly, RNA has been retro transcribed from the polyA tail, and the obtained cDNA is than inserted in a vector and amplified by E. coli. With the same techniques we are also working on the construction of libraries for red tuna and perch.
PCR screening: we have screened the library by PCR, using the library specific primers T3 and T7, (T3: 5’ AAT TAA CCC TCA CTA AAG GG 3’; T7: 5’ TAA TAC GAC TCA CTA TAG GG 3’) to evaluate the sizes of the inserts.

The reactions (in a total volume of 25 µl) were run using 2 µM solution of primers, 2 ml of a selected colon DNA polymerase, 2.5 ml 10X appropriate buffer (Biotools), 0.2 µM dNTPs mix at the following conditions: 94°C for 5 min and 30 cycles at 94°C for 30 sec, annealing at 53°C for 30 sec, elongation at 72°C for 2 min. 4 µl of PCR products were loaded into 1% agarose gel and about 20 µl were sequenced.

Sequencing: by the standardised Sanger method (Amersham Bioscience kit n° US81090) we have sequenced the obtained cDNA fragments starting from the 5’ end.

Bioinformatic analysis: The obtained sequences were analysed by the software Vector NTI (Informax) classified according several parameters and deposited in NCBI Gene Bank (http://www.ncbi.nlm.nih.gov/).

RESULTS AND CONCLUSIONS – At the moment, we have sequenced 283 clones obtained from the library of D. labrax liver. Each sequence has been ordered and analysed by Vector NTI and homologies have been searched by BLASTN and BLASTX in the NCBI databases (http://www.ncbi.nlm.nih.gov/BLAST/). Most of the inserts showed a homology with sequences present in the public data bases. An example of the results obtained for five sequences are shown in Table 1. As reported in Figure 1, about 70% of the examined inserts were longer than 900bp indicating a good quality of the library; 155 out of 283 (55%) were singlets whereas the other 128 sequences (45%) were grouped in 47 contigs.

Table 1. Example of some of the obtained sequences.
| Name       | Blastx analysis | Contig or Singlet |
|------------|-----------------|-------------------|
| 10240_d1_X | gi|38374130|gb|AAR19269.1| (1 element) singlet |
|            | Protein: male specific protein [*Sarotherodon galilaeus*] | |
|            | Score: 188 (Bits: 77.0), Expectation: 4e-013 | |
| 10241_a2_X | gi|1160337|gb|AAA85283.1| (1 element) sin |
|            | Protein: fibrinogen B-beta subunit precursor | |
|            | Score: 368 (Bits: 146.0), Expectation: 4e-034 | |
| 10246_c7_X | gi|677901|gb|AAA62350.1 | (3 elements) sin |
|            | Protein: actin [*Aedes aegypti*] | |