

CONSTRUCTION AND ANALYSIS OF FULL-LENGTH ENRICHED cDNA LIBRARIES FROM NEWLY EXCYSTED JUVENILE OF FASCIOLA HEPATICA

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Fasciola hepatica is the etiological agent of fasciolosis, a worldwide zoonosis that affects mammals. In agriculturally based countries, the infection of ruminants leads to important economic losses (2 billions dollars/year). Our goal is to understand host-parasite relationship and physiological process involved in parasitism in order to identify targets for drugs and vaccines design. For this reason, we started a transcriptomic-based analysis of the invasive stage of *F. hepatica* preparing full-length enriched cDNA libraries from newly excysted juvenile (NEJ). After cDNA synthesis and PCR amplification, we size fractionated the amplification product by agarose gel electrophoresis. Three subpopulations of cDNAs with 300-850bp, 850-1600bp and >1600pb were independently ligated into TA cloning vectors. Colony PCR of randomly picked transformants confirmed the presence of inserts in >90% of the clones. To evaluate the quality of the libraries, plasmid DNA of three 96-well plates was prepared and sequenced using a Megabase DNA analysis system. The resulting sequences were compared to the Genbank, PartiGene and others databases using the tBLASTX program. One hundred seventy-seven high quality sequences were obtained from 288 clones (61%). About 32% had significant similarity (E values < 10⁻⁵) to genes deposited in Genbank database. These sequences are distributed in 31 different clusters constituted by ribosomal protein, antioxidants enzymes (AOXs), mitochondrial enzymes and cysteine proteinases (CPs). A high number of sequences (40%) had no significant similarity to the databases analyzed (40 clusters). This work allowed us to identify genes expressed by NEJ, such as those encoding CPs and AOXs that could be involved in the first steps of the invasion. A high number of hypothetical proteins were detected but their possible role in parasite survival is not known.

Keywords: *Fasciola hepatica*, transcriptome, newly excysted juvenile