

Molecular cloning of cDNA and preliminary analysis of cathepsin L like enzyme from metacercariae of *Fasciola hepatica*

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Fasciola hepatica infections cause significant global problem in veterinary and human medicine. The fluke causes huge losses in cattle and sheep production. Because of its economical importance efforts of many scientists are focused on constructing an effective vaccine against this parasite.

Our approach is to construct vaccine targeting genes crucial for parasite development and pathogenesis. One group of promising vaccine antigens are cysteine proteinases, which play a key role in parasite feeding, migration through host tissues and in immune evasion. However, so far only enzymes from flukes dwelling in final host have been analysed. In the present experiments we aimed to identify cysteine proteinases expressed by the developmental stage of *F. hepatica* infective for the final host.

A total RNA was isolated from metacercariae *F. hepatica* and reverse transcribed. First strand cDNA was synthesized using Reverse Transcriptase and the primer *pTXho*. Based on analysis of variety of cDNA sequences encoding cathepsins L, which are deposited in GenBank database, conserved regions were identified and specific gene primers were designed. The 5' (750 bp) and 3' end (531bp) were amplified using poliG or pTXho and internal specific gene primers by the method of RACE-PCR. Obtained products were cloned into the pBluescript SK+ vector and sequenced using T7 and T3 primers. Sequence analysis showed the highest similarity to procathepsin L from NEJ stage (newly excysted juvenile) of *Fasciola hepatica*. We found also its high similarity to cathepsin Ls from *Fasciola gigantica*. At present a bioinformatics analysis is being performed in order to identify biochemical properties, potential function and structure of the metacercarial cathepsin L.