EXPRESSION ANALYSIS, TRANSCRIPT VARIATION AND RECOMBINANT PRODUCTION OF *SCHISTOSOMA MANSONI* CATHEPSIN D-LIKE ENZYME-2

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Bioinformatics analysis of the *Schistosome mansoni* transcriptome suggested that several cathepsin D-like enzymes (SmCD1-4) may be expressed by distinct evolutive forms. The full SmCD2 transcript has been sequenced before by our group and in this work we aimed at advancing the functional characterization of SmCD2. By RT-PCR on different life cycle stages we detected that the SmCD1, 2 and 3 genes are expressed in adults and in miracidia. Oppositely, only SmCD2 and 3 are expressed in eggs whereas schistossomula exclusively express SmCD2. For transcript variation analysis, the cDNA of SmCD2 was amplified by RT-PCR from the total RNA of adult worms (BH strain). The analysis of 10 clones showed the existence of 8 single-base mutations which can be grouped in five alleles coding for 3 isoenzymes. As suggested by a 3D model of proSmCD2, all 4 missense mutations (Ile245Met, Lys256Glu, Phe322Val and Asp331Glu) can be mapped to surface loops far away from the substrate binding cleft. Using the pET28a vector, the 6x(His)-proSmCD2 protein (~45kDa) could be purified in high-yields from *Escherichia coli* inclusion bodies. A policlonal anti-proSmCD2 serum recognized a major band in adult worm extracts in immunoblots. After unsuccessful attempts to refold the recombinant protein we are now engaged in employing the pMAL-C2X and pGEX-4T1 vectors to express proSmCD2 as soluble fusion proteins in *E. coli*.

Key words: *Schistosomiasis, intraintestinal protein digestion, SmAP gene family, SNPs.*

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