

Production of Recombinant Proteins Containing Immunogenic Epitopes of *Schistosoma mansoni*

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Schistosomiasis is a neglected tropical disease endemic in 78 countries, primarily affecting low- and middle-income regions. Although Praziquantel (PZQ) is available, concerns about drug resistance and limited access to treatment in endemic areas persist. A vaccine would be a valuable addition to the toolbox for control and elimination. Most vaccines tested so far have targeted single antigens, none of which have reached final development. We believe that multi-epitopes vaccines present a promising strategy. Our group previously conducted peptide microarray assays to screen 54 proteins from the tegument, esophageal gland, and gastrodermis, using sera from mice protected by the *Schistosoma mansoni* attenuated cercariae vaccine. This study aims to produce three chimeric proteins: (P1) 20 epitopes from esophageal gland, (P2) 23 epitopes from the tegument, and (P3) 30 epitopes from the gastrodermis. The three synthetic, *E. coli* codon-optimised constructs, which include an AAY spacer between epitopes, were cloned into pET21a vector and expressed in *E. coli* BL21(DE3). The proteins were expressed as inclusions bodies and solubilized using varying concentrations of urea or guanidine. Protein expression was confirmed via SDS-PAGE and Western blot using an anti-His antibody; and purified using nickel affinity chromatography with a linear imidazole gradient. The fractions containing the main protein peak were pooled and stepwise dialyzed/refolded in PBS 1x with decreasing concentrations of chaotropic agents. P1 showed high expression levels, with a yield of 106 mg/L of soluble protein after purification. The tegument protein (P2) had intermediate expression, yielding 29.3 mg/L, but with significant precipitation. The gastrodermis protein (P3) displayed low expression, yielding 1,1 mg/L of soluble protein. The next step is to use these proteins, either individually or in combination, in an immunization and challenge assay using C57BL/6 mice.

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