Evaluation of a novel *Escherichia coli* fusion system for overproduction of recombinant immunogenic proteins

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Recombinant protein production has been widely applied for therapeutic and diagnostic applications, namely for polyclonal antibody production. Antibodies are usually raised against a specific protein by immunization of animals with the purified protein. The bacterium *Escherichia coli* is one of the most used host cells for the bio-production of proteins, but it still presents some drawbacks: many proteins of biomedical interest are difficult to express properly in this host system, resulting in insoluble protein aggregates. Gene fusion technology has been employed to optimize recombinant protein production in *E. coli*. Fusion partners have also been used to potentially increase protein immunogenicity.

In this work, the overproduction and immunopotentiating properties of a novel fusion system were studied. Novel fusion tags, Fh8 and H, were fused to five target proteins with diagnostic interests: CP12, a 12 kDa surface protein from *Cryptosporidium parvum* oocysts; CWP, a cyst wall protein from *Giardia lamblia*; ENT, a surface protein from *Entamoeba histolytica* cysts; TgOWP, a *Toxoplasma gondii* oocyst wall protein; and Frutalin, a recombinant lectin from *Artocarpus incisa* seeds. Production yields of all Fh8-fused proteins, H-fused proteins and non-fused recombinant proteins were compared and polyclonal antibodies were raised against CP12, CWP and ENT non-fused and H-fused antigens.

Overall, the results showed that the fusion of both Fh8 and H tags to all target proteins improved their production in comparison with the respective non-fused target proteins. Moreover, the H tag efficiently increased CP12, CWP and ENT specific immunogenicity without being removed from the fusion antigens and without co-administration of adjuvants, resulting in a more effective and earlier immune response.

The overproduction and immunopotentiating effects observed for this novel fusion system make it a unique alternative for recombinant protein production in *E. coli* and for immunodiagnostic and immunoprophylactic purposes.