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A novel *Escherichia coli* fusion system for production of recombinant immunogenic proteins

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Recombinant protein production is a useful technology 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* has been widely used 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 applied to optimize recombinant protein production in *E. coli*. Fusion partners have also been used to potentially increase protein immunogenicity.

A novel fusion system (Fh8 and H tags) was recently discovered and demonstrated to improve antigen production in *E. coli* [1], presenting also attractive features for the development of antibodies. In this work we explored the immunopotentiating properties of H tag using a 12 kDa surface adhesion protein of *Cryptosporidium parvum* (CP12) [2] as model antigen. Production yields of Fh8CP12, HCP12 and CP12 recombinant proteins were evaluated and polyclonal antibodies were raised against both CP12 and HCP12 antigens. Results obtained here demonstrated that the fusion of both Fh8 and H tags to CP12 improved its expression in comparison with non-fused CP12 protein, and that H tag efficiently increased CP12 specific immunogenicity without being removed from the fusion antigen and without co-administration of adjuvants, resulting in a more effective and earlier immune response.

References

[1] Castro A, Almeida A, Costa S, Conceição M, "Fusion proteins, its preparation process and its application on recombinant protein expression systems", (2009) PCT/IB2009/055647.
[2] Yao L, Yin J, Zhang X, Liu Q, Li J, Chen L, Zhao Y, Gong P, Liu C, "*Cryptosporidium parvum*: identification of a new surface adhesion protein on sporozoite and oocyst by screening of a phage-display cDNA library", *Exp Parasitol*. (2007) 115: 333-338.