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DEVELOPMENT OF A NOVEL FUSION SYSTEM FOR SOLUBLE PROTEIN OVEREXPRESSION AND PURIFICATION IN *ESCHERICHIA COLI*

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Recombinant proteins, *Escherichia coli*, fusion system, overexpression

ABSTRACT

Recombinant protein production is a useful technology for therapeutic and diagnostic applications. The bacterium *Escherichia coli* is widely used for the bio-production of proteins, but still presents some drawbacks. Recombinant proteins with biomedical interest have proved difficult to express properly in this host system, resulting in insoluble protein aggregates. Fusion protein technology has been applied to overcome these drawbacks and to optimize protein expression in *E. coli*. This research aims at the evaluation of a novel fusion system (Fh8 and H tags) effects on protein production. Target proteins used in this work present biomedical interest and results obtained here showed that these novel fusion tags increased protein production yields. This novel fusion system is a promising tool for the production of recombinant proteins with biomedical interest.

INTRODUCTION

Recombinant protein production had become a valuable technology for the development of biomedical solutions. A range of host cells and expression systems have been used for recombinant protein expression, including *Escherichia coli* – the main widely used factory for bio-production of proteins. In spite of offering a rapid and low cost production of the desired protein, many proteins of biomedical interest have proved difficult to express properly in this host system, resulting in insoluble and instable protein aggregates, known as inclusion bodies (Jana and Deb 2005; Terpe 2006). Fusion protein technology has been applied to optimize protein expression in *E. coli*. The ideal fusion partner/tag should increase target protein production yields, promote its solubility and help on its purification (Waugh 2005; Esposito and Chatterjee 2006). Several fusion partners had already been described in literature; most of them are well employed in laboratories around the world. Until now, however, none of these fusion systems provided an efficient and universally effect on target protein production, bringing with it a new set of opportunities for improvement. A novel fusion system with the potential for soluble protein overexpression in *E. coli* was recently discovered (Castro, et al. 2009). This system consists of two new fusion partners: Fh8 and H tags, which are characterized by their low molecular weight (8 kDa and 1 kDa, respectively) and their ability to improve the production of difficult-to-express proteins. Taking into account the potentialities mentioned above, this research aims at the evaluation of the novel fusion tags effects on recombinant protein production in *E. coli*. First, it is intended to explore the application of Fh8 and H molecules as novel fusion partners and latter, to discover the mechanisms behind the stability and solubilisation actions potentially conferred by the new fusion partners. Different target recombinant proteins with biomedical applications are being used to complete the first goal. These target proteins were previously produced in *E. coli* but remained difficult-to-express on this host cell, presenting low production yields. This work reports the effect of the novel fusion partners on production yields of *Toxoplasma gondii* oocyst wall protein (TgOWP) and *Entamoeba histolytica* cyst wall protein (ENT). Both recombinant proteins present a diagnostic and therapeutic potential.

METHODS

Fusion DNA construction

Target protein codifying sequences were amplified from genomic DNA by PCR, using specific primers to introduce the recognition sites for restriction enzyme cleavage. PCR products were cloned into pGEM T-easy vector (Promega) and used to transform *E. coli* XL1 strain. Positive clones were selected and digested with specific restriction enzymes to be inserted next into fusion vectors containing Fh8 or H tags. *E. coli* M15 [pREP4] cells were then transformed with the resulting fusion vectors and used for fusion protein expression.

Fusion proteins production

E. coli cells were grown at 37 ºC in LB medium supplemented with specific antibiotics. When the O.D.$_{600nm}$ reached 0.5, cells were induced with 1 mM of IPTG for 5 hours at 37 ºC for the expression of recombinant fusion proteins. Cells were then harvested by centrifugation and the resulting pellets were lysed overnight with 8M urea. After cell extracts centrifugation, fusion protein soluble fraction was purified by nickel affinity. The produced fusion proteins were analysed by SDS-PAGE and quantified by Bradford assay.

RESULTS

In order to access the effect of Fh8 and H novel fusion tags on target proteins production, a comparative study was conducted between non-fused proteins and fusion proteins with Fh8 and/or H tags.

When fused to H-tag, TgOWP recombinant protein was successfully produced in *E. coli*. The fusion of H tag promoted a production yield 6-folds higher than the obtained for non-fused protein (Figure 1.a). SDS-PAGE analysis confirmed the production of both TgOWP (13 kDa) and H-TgOWP (14 kDa) proteins at the expected molecular weight (Figure 1.b).

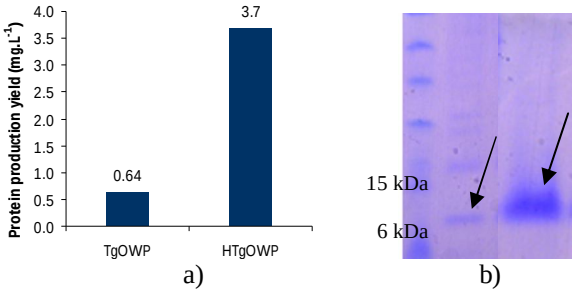

Figure 1. Production yields obtained for TgOWP and H-TgOWP recombinant proteins (a) and proteins SDS-PAGE analysis (b).

A similar effect was observed for ENT recombinant protein. The addition of both Fh8 and H tags resulted in a production yield 3-folds higher than the obtained for non-fused ENT protein (Figure 2.a). SDS-PAGE analysis showed that non-fused (7 kDa) and fused ENT (H-fused 8 kDa; Fh8-fused 15 kDa) proteins were produced at the expected molecular weights (Figure 2.b).

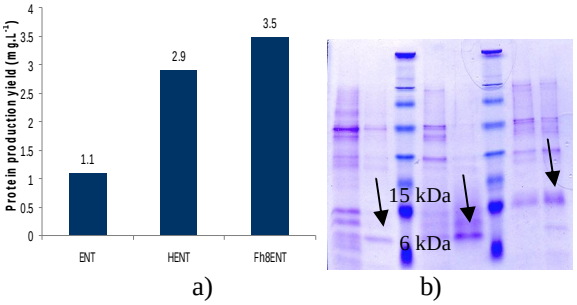

Figure 2. Production yields obtained for ENT and H-ENT recombinant proteins (a) and proteins SDS-PAGE analysis (b).

CONCLUSIONS

This work is still in progress. Results presented here demonstrated that both novel fusion partners - Fh8 and H tags - promoted an increase of protein production yields in *E. coli*, making them promising tools for the production of recombinant proteins with biomedical interest. Nevertheless, we have new goals: to access the effect of novel fusion tags on target protein biological activities, to improve purification methodologies and finally, to understand the mechanisms behind the overproduction effect inside the host cell.

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