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EVALUATION OF A NOVEL ESCHERICHIA COLI FUSION SYSTEM FOR THE PRODUCTION OF RECOMBINANT IMMUNOGENIC PROTEINS

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KEYWORDS
H fusion partner, immunogens, polyclonal antibodies.

ABSTRACT

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 immunisation of animals with the purified protein. The *Escherichia coli* host cell is widely used for the bio-production of proteins with biomedical interest but some of them are still difficult to express properly in this host system, resulting in insoluble protein aggregates. Gene fusion technology has been employed to optimise recombinant protein production in *E. coli*. Fusion partners have also been used to increase protein immunogenicity. In this work, the immunopotentiating properties of a novel fusion partner (H partner) were studied. The H partner was fused to three target proteins with diagnostic interest: CP12, a 12 kDa surface protein from *Cryptosporidium parvum* oocysts; CWP, a cyst wall protein from *Giardia lamblia*; and ENT, a surface protein from *Entamoeba histolytica* trophozoites. The results obtained here show the H partner as a promising tool for immunodiagnostic and immunoprophylactic purposes.

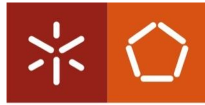
INTRODUCTION

Recombinant protein production became a valuable technology for the development of biomedical solutions. The host cell *Escherichia coli* has been widely used for the 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 protein aggregates, known as inclusion bodies (Jana and Deb 2005; Terpe 2006). When using these recombinant proteins (antigens) for polyclonal antibody production, most of them fail to elicit an immune response, requiring adjuvants for immunisation (Knuth, et al. 2000). Fusion protein technology has been applied to optimise antigen expression in *E. coli* and also to improve the polyclonal antibody production. Several fusion partners had already been used for antigen production and immunisation, namely, GST and MBP (Kink, et al. 1998; Lopez-Monteon, et al. 2004). In spite of the antigen production improvements, these fusion partners often required adjuvants for immunisation. In addition, due to the large size of fusion partners, the produced polyclonal antibodies were not antigen-specific, reacting against both antigen and fusion partner. A novel fusion partner of 1 kDa for antigen immunisation, the H partner, is presented in this work. The immunpotentiating properties of the H partner were studied using three antigens with diagnostic and therapeutic potential (CP12, CWP and ENT). The immune responses obtained were investigated and a diagnostic application of the produced polyclonal antibodies was explored.

METHODS

Recombinant antigen production

The genes that codify for CP12, CWP and ENT antigens were previously cloned into the pQE H plasmid and into the pQE 30 plasmid. *E. coli* M15 [pREP4] 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. Supernatant fractions were prepared for immunisation.



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Polyclonal antibody production

Polyclonal antibodies against the H-fused antigens and non fused antigens were produced in CD1 female mice. Three groups of mice were injected intraperitoneally at 2 week intervals with the studied antigens, without using adjuvants. Blood collection was carried out periodically for five times. The mice sera was treated and used for ELISA and immunofluorescence assays (IFAs). All animal experiments were conducted accordingly to the European Communities Council Directive (86/609/EEC).

RESULTS AND DISCUSSION

The expression of all fusion antigens was analysed by SDS-PAGE and all the proteins presented a molecular weight identical to the expected, calculated by the ProtParam Expasy tool (data not shown). The polyclonal antibody productions were analysed and compared by ELISA. The CP12 and CWP fusion antigens elicited an earlier and higher humoral response than the non fused ones (Fig. 1.a and 1.b). The polyclonal antibody production against the ENT antigen was not evaluated. Nevertheless, an humoral response was elicited by the fusion antigen HENT, as it can be observed in Fig. 1.c.

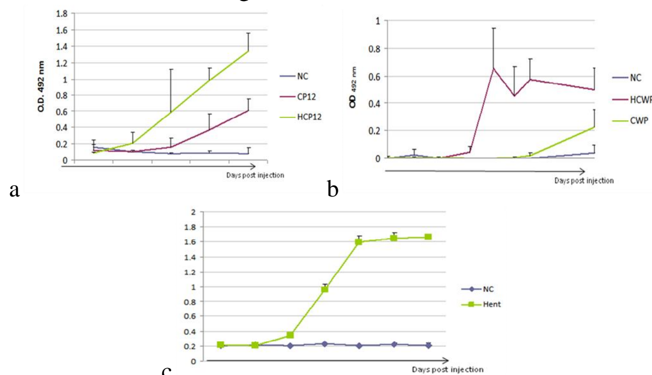

Figure 1. ELISA results of the produced polyclonal antibodies. NC – negative control mice group.H"Antigen" – mice group injected with the fusion antigens or "Antigen" – with the non-fused ones.

The potential application for diagnosis of the respective parasite infections in animals was studied by IFAs using the polyclonal antibodies produced against the fusion antigens. The anti-HCP12 polyclonal antibodies detected the native epithopes on the surface of *Cryptosporidium parvum* oocysts (Fig. 2.a). The anti-HCWP polyclonal antibodies presented a similar detection result, identifying the native epithopes on the surface of *Giardia lamblia* cysts (Fig. 2.b). The anti-HENT polyclonal antibodies were able to detect the native parasite structures exhistent on a commercial plate of *Entamoeba histolytica* trophozoites (Fig. 2.c).

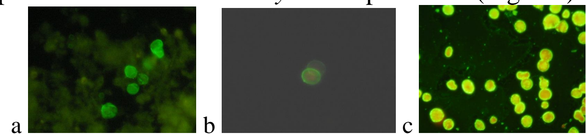

Figure 2. Detection of the native parasite structures using the produced polyclonal antibodies: a) anti-HCP12, b) anti-HCWP, c) anti-HENT.

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