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ARTICLE

Cloning and expression of FimA-c3d recombinant protein

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Abstract Recombinant protein consisting of an antigen fused to C3d may elicit a more robust immune response than the antigen alone. The objective of the present study was to clone FimA-c3d recombinant DNA into pCold-TF vector and successfully express in prokaryotic expression vector. FimA subunit of type I fimbriae from *Salmonella enterica* serovar Enteritidis was conjugated to mouse complement fragment molecule *C3d*. FimA and FimA-*mC3d*, FimA-$mC3d_2$ and FimA-$mC3d_3$ were cloned into the expression vector pCold-TF. Constructions of the recombinants pCold-TF-fimA with different copies of *C3d* were confirmed by digestion with restriction enzymes and sequencing. Soluble fusion proteins of FimA with different copies of *C3d* were induced by IPTG and were expressed in *Escherichia coli* BL21 (DE3) under optimal conditions. The results showed that the proteins induced from recombinants pCold-TF-fimA, pCold-TF-fimA-*mC3d*, pCold-TF-fimA-$mC3d_2$ and pCold-TF-fimA-$mC3d_3$ were 70, 100, 130 and 160 kDa, respectively. The fusion protein was recognized by rabbit anti-fimbriae polyclonal antibodies, and then visualized by goat anti-rabbit polyclonal antibodies with a chrome appearance by enzyme-subtract interaction. The recombinant proteins were separately purified by Ni-TED (tris-carboxymethyl ethylene diamine) immobilized metal iron affinity chromatography (IMAC). Finally we conclude that antigen conjugated with mC3d can be functionally expressed in prokaryotic vectors.

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1. Introduction

Fimbriae of *Salmonella enterica* serovar Enteritidis are used for colonization and invasion into host cells, and have drawn considerable interest because fimbriae can serve as potential immunogens against many pathogenic bacteria that colonize epithelial surfaces [24]. The main fimbriae of the *S. enterica*

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serovar Enteritidis are SEF14, SEF17 and SEF21 composed of SefA, AgfA and FimA fimbrial proteins, respectively [13]. Fimbria-based vaccines are hypothesized to protect the host against the adherence of pathogens by blocking the attachment of organisms to the intestinal mucosa [31]. Recently, scientist's interest has revolved around the effecter molecules generated by the innate response and their role in shaping acquired immunity [6]. Recombinant protein consisting of an antigen fused to *C3d* may elicit a more robust immune response than the antigen alone. Dempsey et al. [2] demonstrated that a recombinant protein containing three copies of *C3d* attached to the carboxy terminus of hen egg lysozyme (HEL) could elicit a primary immune response at a concentration 10,000-fold more than that required for the unmodified HEL protein.

A major bottleneck in structural biology is that many human proteins are expressed very poorly or are expressed well but not in soluble forms [14]. These difficulties may arise due to the improper folding of human proteins in *Escherichia coli* cells, where they are quickly digested by proteases or accumulated as inclusion bodies [14]. A number of expression systems have been developed to overcome this problem, for example, by coexpressing human proteins with molecular chaperones [21], expressing them as fusions with maltose-binding protein [4], glutathione *S*-transferase [27], or expressing them at low temperatures using cold shock vectors [23]. Heat-shock response from bacteria to humans has been extensively studied, while cold-shock response has caught the attention of researchers relatively recently [22]. A major reason why heat shock is extensively studied is because it causes well-defined damage to the cells, i.e. unfolding or denaturation of proteins. In contrast, cold shock does not cause such well-defined cellular damage. Cold-shock response is classically exhibited when an exponentially growing culture is shifted from its optimum growth temperature to a lower temperature [22]. Several cold shock proteins have been detected not only in *E. coli* but also in many other bacteria [17,30,25,10]. Certain aspects of bacterial cold-shock response such as regulation of expression of CspA homologues have been extensively reviewed [5,9,29,35]. CspA function was proposed to be an RNA chaperone [35,8]. The CspA family is essential for *E. coli* cells to adapt to low temperature [32,3]. A peptidyl prolyl isomerase (trigger factor-TF), that catalyzes the *cis*/*trans* isomerization of peptide bonds N-terminal to the proline residue was identified in *E. coli*. This enzyme is induced upon cold shock at a modest level after a growth lag period of 2–3 h, its overexpression leads to enhanced viability [12]. The objective of the present study was to clone FimA-c3d recombinant DNA into pCold-TF vector and successfully express in the prokaryotic expression system.

2. Materials and methods

2.1. Strains and vectors

Standard strains of *Salmoenlla enterica serovar Enteritidis* SD-2, *E. coli* DH5α and recombinant protein expression recipient strain *E. coli* BL21 (DE3), PCold-TF expression vector and recombinant plasmid pUC-mC3d, pUC-mC3d2 and pUC-mC3d3 and *Salmonella Enteritidis* -type pilus polyclonal (rabbit) were preserved in our laboratory. Primers were synthesized by Shanghai Gene Core Biotechnology Co., Ltd., and pMD18-T. Simple vector was purchased from Takara Biotechnology Co., Ltd.

2.2. Reagents

Restriction enzymes NdeI, BamHI, BglII and T4 DNA ligase were purchased from NEB's, Amp (Ampicillin) and Expand High Fidelity PLUS PCR System was purchased from the Roche Company, Purification of Polyhistidine-Tagged Proteins (Proteins® Ni-TED2000) were purchased from MACHEREY-NAGE company, DNA marker and lysozyme were purchased from the Takara Company, High molecular weight marker and Pre-stained low molecular weight marker were purchased from the Fermentas Company, 3,3′-diaminobenzidine (3,3′-diamino benzidine, DAB) and agarose gel DNA extraction kit (centrifugal columnar) were purchased from the BBI (Bio basic Inc.) company, Isopropyl-β-D-thiogalactoside (IPTG) were purchased from Takara Biotechnology Co., Ltd., goat anti-rabbit HRP-IgG antibodies were purchased from the Boster Company, sodium dodecyl sulfate (SDS), ammonium persulfate (APS), N,N,N,N-tetramethylethylenediamine (TEMED) is from the AMRESCO company, electrophoresis grade acryl amide and Tris-base were from BioSharp, and N,N-methylene-bis-acrylamide was from Gibco.

2.3. Bacteria growth and DNA extraction

Salmonella enterica serovar Enteritidis was cultured over night at 37 °C with vigorous agitation and then DNA was extracted. *FimA* gene (6949132) was amplified by an upper primer with NdeI restriction enzyme site: 5'-CGC CATATG AAA CAT AAA TTA ATG ACC TCT A-3' and lower primer with NotI and BamHI restriction enzymes site: 5'-TCG GCG GCC GCG GAT CCT TCG TAT TTC ATG ATA AAG GTG-3'. PCR was carried out in a total volume of 25 μl containing 2.5 μl DNA template, 1.5 μl of 2.5 mM dNTP mixture, 2.5 μl of 10 × PCR buffer, 1 μl upper primer, 0.5 μl lower primer, 0.5 μl Ex Taq polymerase and 16.5 μl sterilized distilled water. PCR conditions were initial denaturation at 94 °C for 4 min, followed by 30 cycles at 94 °C for 1 min, annealing at 56 °C for 1 min and extended at 72 °C for 1 min, and finally the PCR product was extended at 72 °C for 10 min. The 550 bp of *FimA* gene was excised from the gel and purified using DNA purification kit. Purified product was then cloned into pMD18-T simple vector and transformed into *E. coli* DH5a competent cells. The *E. coli* DH5a carrying recombinant plasmid (pMD18-T + *FimA*gene) was grown on LB plates containing 100 μg/ml ampicillin at 37 °C overnight, and the single clone was grown in 5 ml LB broth plus ampicillin (100 μg/ml) at 37 °C to OD600 of 0.4–0.5. Recombinant plasmids were extracted and digested by restriction enzyme and sequenced.

2.4. Construction of recombinant protein

Recombinant plasmids pMD18-T-FimA and pCold-TF vectors were digested with NdeI and BamHI restriction enzymes. Both *fimA* gene and pCold-TF expression vector were recovered from the gel using the agarose gel DNA purification kit. Purified fimA DNA was cloned into pCold-TF expression vector (Fig. 1). The recombinant pCold-TF-fimA was transformed into the *E. coli* DH5a competent cells; positive clones were selected and determined by restriction enzyme NdeI and BamHI. Clones of