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Expression of the constant region of bovine IgG1 heavy chain in *E. coli*

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ABSTRACT

Nejad AJ, Ashtary A, Ghorbanpoor M, Seyfi M, Shapouri A., Expression of the constant region of bovine IgG1 heavy chain in E. coli, Online J Vet Res., 17 (1): 28-33, 2013. The aim of present study was to verify if the constant region of bovine IgG1 heavy chain in *E. coli* could be expressed to produce anti-antibody against bovine IgG1. Specific primers were designed to amplify the constant region of G1 chain mRNA by RT-PCR. The resulting DNA fragment was cloned in pMal-C2X expression plasmid and expressed in TG1 and BL strains of *E. coli*. Expression of an expected fusion protein (MBP-G1), comprising of a Maltose binding protein (MBP), encoded by the plasmid and the constant region of G1 chain (CH1-CH3) was confirmed by SDS-PAGE and Western blot. The protein was found to be insoluble in BL21(DE3) strain but partially soluble in TG1, when it was expressed at room temperature.

Key Words: Bovine, IgG1, constant region, expression, *E. coli*

INTRODUCTION

Diagnosis of infections caused by pathogenic micro organisms usually involves the use of immunological tests to detect antibodies specific for these pathogens. In most of immunological tests, including immunohistochemical and immunoenzymatic assays, the

reaction of antibodies with their targets, the antigens, are revealed by secondary, conjugated anti-antibodies. Anti-antibodies are poly or monoclonal antibodies, labeled with various enzymes or dyes, depending on the assays.

Most frequently, the anti antibodies are obtained from blood of an experimental animal (rabbit, goat, sheep or donkey) immunized with a purified antibody preparation. Purification of serum immunoglobulin, specially a given immunoglubulin class or subtype is time consuming and sometimes a difficult procedure.

Expression of the constant region of immunoglobulin chains in a laboratory host (Kenten *et al.,* 1984*;* Nakamura *et al.,* 1988) could be an alternative procedure to obtain a pure and even an isotype specific antigen for immunization. Among the laboratory expression systems, cloning and expression of genes in *E. coli* is the most simple and a rapid way for recombinant proteins production.

Cattle have three IgG subclasses: IgG1, IgG2 and IgG3. IgG1 constitute about 50% of serum IgG and is remarkable for being the predominant immunoglobulin in cow's milk rather than IgA (Tizard, 2004)). The purpose of present study was to verify the possibility of expression of the constant region of bovine IgG1 heavy chain in *E. coli*, for production of anti-antibody against bovine IgG1 in future studies.

MATERIALS AND METHODS

RT-PCR

Based on the nucleotide sequence of bovine immunoglobulin G1 chain (X62916.1), specific primers(Forward: 5 - GCCGAAGCTTTTAATTGTCATACTCCTCTGCATTGT -3 and Reverse: 5'-GCCGGAATTCATGGCGTCTCAAGGCAC - 3 ') were designed to amplify the constant region (CH1-CH3) of the chain by RT-PCR. Primers included recognition sites for the restriction enzymes *EcoR I* and *Sal I* for cloning of the RT-PCR product in the pMAL-C2X plasmid (New England Bio Labs, USA).

As a source of mRNA of G1 chain, small pieces of fresh spleen tissue of a slaughtered bull were immediately harvested and transferred to the laboratory. RNA was extracted by Tripure reagent (Roche, Germany), according to manufacturer instructions and dissolved in diethyl pyrocarbonate (Sigma, USA) treated water. cDNA was synthesized using Revertaid enzyme (Fermentas, litvany) and the reverse primer at 42ºC and amplified in PCR through 30 cycles of 94ºC 1 min, 45.8°C 1 min and 72°C 1.5 min.

Cloning of the constant region of bovine G1 chain in *E. coli*

The product of PCR with a length of 1010 bp was purified after electrophoresis on agarose gel, using a commercial gel extraction kit (Qiagen, Germany). The purified PCR product and the pMAL-C2X plasmid, both were digested with *EcoR I* and *Sal I* (Fermentas, Litvany) restriction enzymes, electerophoresed on a 1 percent agarose gel

and purified again by the gel extraction kit. Thereafter, the digested PCR product was ligated into the plasmid by T4 ligase during an overnight incubation at 4ºC. The new construct, was transformed into *E. coli* TG1 host strain by a one step transformation method (Chung *et al.,* 1989). Bacterial colonies appeared on LB agar supplemented with ampicillin were screened and a positive clone was selected for expression of the recombinant fusion protein (MBP-G1). MBP is a maltose binding protein which is encoded by the pMAL-C2X plasmid (Riggs, 2000).

Expression of MBP-G1 fusion protein

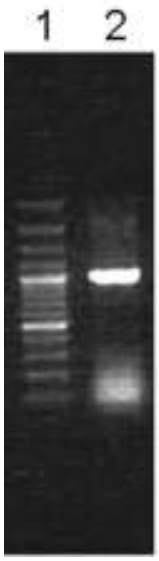
The selected positive clone was subjected to plasmid extraction by a commercial plasmid extraction kit (Qiagen, Germany) and then the purified plasmid was transformed into *E. coli* BL21(DE3) strain for expression of the fusion protein.

Bacteria were grown overnight in 5 mL LB broth supplemented with ampicillin at 37ºC,. Ten ml of LB broth supplemented with ampicillin and 20mM glucose was inoculated with 100 µl of the overnight culture and incubated at 37ºC, until the OD_{600} of bacterial culture was 0.5. At this point a 1 ml sample of bacteria was collected, centrifuged at 13000 xg for 2 min and the harvested cells were stored at -20ºC. Then, expression was induced in the remaining culture by adding 0.3 mM IPTG. Four hours after induction, a 0.5 ml sample was collected and processed as described. The cells harvested before and after induction were resuspended in SDS-PAGE sample buffer, boiled 5 min and analyzed on a 10% polyacrylamide gel, followed by Coomacie brilliant blue staining. The recombinant fusion protein was also detected by Western blotting using a commercial anti bovine IgG conjugated with peroxidase (Sigma, USA). After SDS-PAGE of the samples, proteins were transferred on a nitrocellulose membrane at 12 V for 3 hours. The membrane was blocked with 5% horse serum in phosphate buffered saline-Tween 20 (PBST: PBS containing 0.05% Tween20) for 2 hours and then probed with 1/1000 dilution of the peroxidase conjugate in PBST, containing 2% horse serum. Subsequently the membrane was washed three times with PBST, 5 min each and developed by 4-chloro-1-naphtol.

In order to determine the solubility of the fusion protein, expression of the protein in BL21(DE3) strain was induced by IPTG as described above, for 4 hours and at 2 temperatures of 37ºC and 25ºC. After induction, the bacterial cells were collected by centrifugation and resuspended in PBS. The cells were broken by sonication and centrifuged at 18000 xg, 20min at 4ºC. The pellets and supernatants were analyzed along side each other by SDS-PAGE as the insoluble and soluble proteins respectively. A similar process of the solubility assay was also carried out for the protein expressed in TG1.

RESULTS

RT-PCR with the designed primers resulted in amplification of an expected DNA band of 1010 bp (Fig 1). After cloning of the PCR product in plasmid pMAL-C2X, a clone of the gene in *E. coli* BL21(DE3) strain was selected and induced for expression of the fusion protein (MBP-G1).



·Fig 1. Amplification of the constant region of bovine IgG1 heavy chain mRNA by RT-PCR. (Lane 1: 100 bp DNA ladder; Lane 2: RT-PCR product)

SDS-PAGE analysis of bacterial proteins after induction indicated the expression of a fusion protein with the expected molecular weight of 79 kD (Fig 2a). Positive reaction of the expressed protein with a commercial peroxidase conjugated anti bovine IgG in immunoblot confirmed its identity as part of Bovine IgG molecule (Fig 2b).

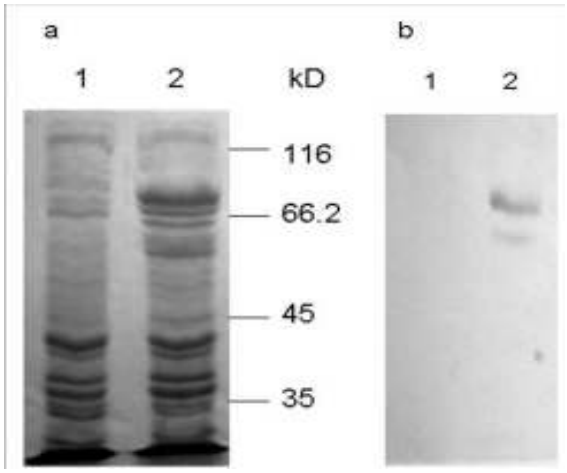


Fig 2. Expression of MBP-G1 fusion protein, in *E. coli*, BL21(DE3) strain, analyzed by SDS-PAGE (a) and immunoblot (b). Lanes 1 and 2 represent the bacterial proteins before and after induction respectively. Molecular weights (kD) are indicated at the right of Fig 2a.

While the protein expressed in BL21(DE3) strain was insoluble (Fig 3A), it was found to be partially soluble in TG1 (Fig 3B), when it was expressed at room temperature (25ºC) for 4 hours.

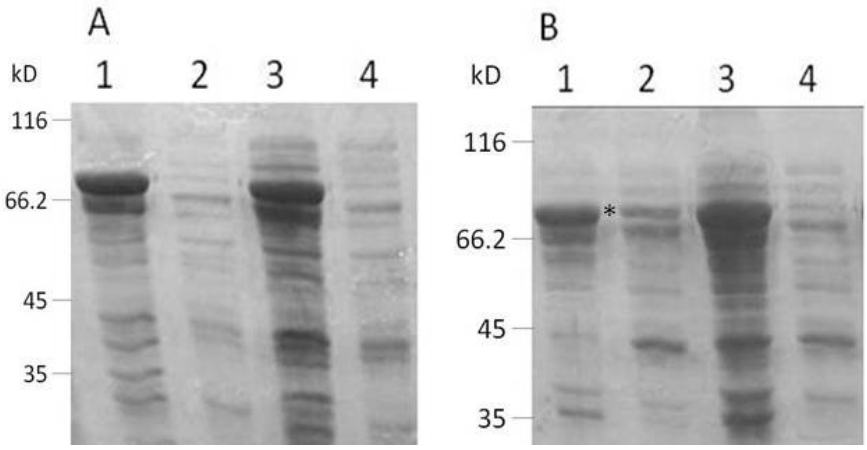


Fig 3. Solubility of MBP-G1 fusion protein in *E. coli*, BL21(DE3) (A) and TG1 (B) strains, after induction for 4 hours at 25°C (lanes 1 and 2) and 37°C (lanes 3 and 4). Lanes 1 and 3 at both figures represent the insoluble and lanes 2 and 4 correspond to soluble proteins respectively. The soluble form of the fusion protein expressed at 25°C in TG1 is indicated by an asterisk (*).

DISCUSSION

In the present study we have investigated the utility of *E. coli* for expression of the constant region of bovine IgG1 heavy chain. Therefore, coding region of interest was RT-PCR amplified, ligated to pMal-C2X expression vector and expressed in BL21(DE3) and TG1 strains of *E. coli*. The pMal-C2X vector enables the N-terminal fusion to a cleavable MAL-Tag sequence, encoding a maltose binding protein (MBP). Despite a widespread use of the MBP tag to increase the solubility of *E. coli* expressed recombinant proteins (Kapust and Waugh, 1999), we found the fusion protein produced in BL21(DE3) strain was insoluble. Protein solubility is a common problem in recombinant protein expression. Proteins have generally the highest solubility in their natural environments, because a given natural environment provides all materials and tools necessary and sufficient for a particular protein expression.

In practice, many factors affect the solubility of recombinant proteins. Medium composition, rate of protein synthesis, cellular location of expression and the host in which the protein is expressed are among the important factors which affect the protein solubility (Betts and King, 1999; Hockney, 1994; Schumann and Ferreira, 2004). In present study we have evaluated the effects of factors like temperature and host on solubility of the recombinant protein. It was noted that the protein was partially soluble in TG1 strain of *E. coli*, when induction performed at room temperature for 4 hours. This condition could decrease the rate of protein synthesis and therefore increase the protein solubility. Differences in expression of cellular tools or chaperons could account for solubility of the protein in TG1 compared to BL21(DE3) strain.

The overall results of this research indicate that the constant region of bovine IgG1 heavy chain can be expressed as a partially soluble fusion protein in *E. coli*.. This strategy is simple and when established, eliminates the time consuming steps of immunoglobulin purification. Moreover, by this strategy it is possible to express the isotype specific sequences of immunoglobulins and facilitate the production of isotype specific anti antibodies.

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