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Molecular Cloning, Expression and Purification of Fructose-1, 6-bisphosphate Aldolase of *Neisseria meningitidis*-MC58

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Abstract: *Neisseria meningitidis* is an exclusively human nasopharyngeal commensal, which remains a leading cause of life-threatening meningitis and septicemia. Fructose-1, 6-bisphosphate aldolase (FBA) is a cytoplasmic glycolytic enzyme, which despite lacking identifiable secretion signals have also been shown to be transported to the surface of numerous bacteria and shown to possess diverse, non-glycolytic biological functions. *In silico* analysis of FBA sequences from *N. meningitidis* and *N. gonorrhoeae* shows a high degree of sequence conservation at the amino acid level, but only low-level (*ca*. 30%) homology to the FBA homologue from *S. pneumoniae*. The gene encoding FBA from *N. meningitidis* serogroup B strain MC58 was cloned in two different vectors carrying an N-terminal and C-terminal His-tags and expressed in host *E. Coli.* Attempts to express and purify the recombinant protein with an N-terminal fusion tag yielded mostly insoluble and inactive protein. However, the *ca*. 38-kDa recombinant protein corresponding to FBA was affinity-purified under denaturing conditions with N-terminal fusion tag and non-denaturing conditions with C-terminal fusion tag to near homogeneity and used to raise polyclonal monospecific rabbit serum to facilitate subsequent characterization of the enzyme. The molecular mass of the recombinant FBA was in agreement with the mass calculated on the basis of its gene sequence.

Keywords: *Neisseria Meningitidis*, Aldolase, Cloning, Over-Expression, Purification

1. INTRODUCTION

Glycolytic enzymes, along with other enzymes involved in central metabolism are sometimes called 'housekeeping enzymes'. These classical cytoplasmic proteins that are known to be constitutively expressed and localised mainly to the cytoplasm of the cell. There is growing evidence that several so-called housekeeping enzymes, including fructose bisphosphate aldolase (FBA) and glyceraldehydes-3-phosphate dehydrogenase (GAPDH), are often localised to the surface of bacterial pathogens. Whilst their intracellular functions are well known, it is unclear whether they perform any additional functions on the bacterial surface. *Neisseria meningitidis* remains an important cause of septicemia and meningitis and is associated with high morbidity and mortality (Stephens *et al.*, 2007). As an obligate human commensal, it colonizes the nasopharyngeal mucosa of a substantial proportion of the population in an asymptomatic manner. In susceptible hosts, hyper-invasive strains of meningococci possess the ability to invade the nasopharyngeal sub-mucosa and enter the bloodstream, where they can multiply rapidly to high levels. *N, meningitidis* may also cross the brain vascular endothelium, proliferate in the cerebral-spinal fluid (CSF) and cause meningitis (Stephens, 2009).

Fructose 1, 6-bisphosphate aldolase catalyses the reversible cleavage of fructose 1,6-bisphosphate into two triose sugars: dihydroxyacetone phosphate (glycerine-P) and glyceraldehyde 3-phosphate in the glycolysis cycle (Ramsaywak *et al.*, 2004). Besides performing a key role in glycolytic cycle, aldolases have also been shown to be localised to the surface of numerous bacterial and fungal pathogens. In *S. pneumoniae* surface-localized FBA has been shown to bind the host cell ligand Flamingo cadherin (Blau *et al.*, 2007), and also found to be a surface-localized immunogenic protein in *S. suis* (Zongfu *et al.*, 2008). Thus, FBA may be multifunctional protein with a role in central metabolism and an additional role unconnected to its primary role which is associated with an alternative sub-cellular localisation. Such proteins have been described as 'moonlighting proteins' (Jeffery, 1999; Jeffery, 2009)**.** The aim of the present study was to clone, over-express *fba* and subsequently to affinity purify full length recombinant FBA. The purified protein was then used to raise polyclonal antiserum against the purified proteins to facilitate subsequent functional and molecular characterisation studies of the enzyme.

2. MATERIALS AND METHODS

2.1 Bacterial strains, growth conditions, and media

E. coli strains and plasmids used in this study are described in **(Table 1).** *E. coli* strains were routinely grown at 37°C in LB broth or on LB agar. Where appropriate, antibiotics were used at the following concentrations: ampicillin (100 µg ml^{-1}), kanamycin (30µg ml^{-1}). Where appropriate, blue/white selection of transformants in cloning experiments was achieved

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using IPTG (Roche) and X-gal at 0.5 mm and 80 µg ml^{-1} concentrations, respectively. All liquid cultures were aerated by agitation at 200 revolutions per minute (rpm) in a shaking incubator.

Table 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Description	Source or reference
***E. coli* strains**		
JM109	*end*A1 *rec*A1 *gyr*A96 *thi hsd*R17 ($r_K^-r_K^-$) *rel*A1 *sup*E44 Δ(*lac-pro*AB) [F′ *tra*D36 *pro*AB *laq*I^{q}ZΔM15]	Promega
TOP10F′	F′*lac*Iq*Tn*10(TetR) *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L *end*A1 *nup*G	Invitrogen
BL21(DE3)pLysS	F- *ompT hsd*S (rB-mB-) *gal dcm* (DE3) pLysS (CamR)	Invitrogen
Plasmids		
pQE70	Expression vector encoding resistance to ampicillin	Qiagen
pEXP/NT-TOPO	Expression vector encoding resistance to ampicillin	Invitrogen
pSAT-1	MC58 *cbbA* gene cloned in pEXP/NT-TOPO	This study
pSAT-9	MC58 *cbbA* gene cloned in pQE70	This study

Table 2. List of primers used in this study

Primer	DNA sequence [a]	Restriction site
FBA_E1(F)	CGC**GGATCC**ATGGCACTCGTATCCATGCG	*BamH*I
FBA_E2(R)	CGC**GGTACC**GTCGTCCGAACGGCGG	*Kpn*I
FBA_pQE70(F)	CGC**GCATGC**ATGGCACTCGTATCC-3'	*Sph*I
FBA_pQE70(R)	CGC**AGATCT**TTTGACGATTTGGTTCAA-3'	*Bgl*II

[a] All primers were designed from the *N. meningitidis* MC58 genome sequence. Sequences in bold identify restriction enzyme sites.

2.2 DNA manipulation

Chromosomal DNA of *N. meningitidis* was extracted by using a DNeasy Blood & Tissue kit (Qiagen UK.) using the protocol for bacterial cells recommended by the manufacturer. The plasmid DNA was purified from 5 ml overnight cultures of respective *E. coli* strains using a QIAprep spin kit (Qiagen UK) according to the manufacturer's instructions. Restriction endonuclease enzymes and T4 DNA ligase were purchased from Roche, UK. All enzymatic reactions were carried out according to the manufacturer's instructions. The concentration of purified plasmid and genomic DNA was quantified using a NanoDrop (ND-1000) spectrophotometer (Agilent Technologies UK). DNA sequencing was carried out at the School of Biomedical Sciences (University of Nottingham) on an ABI 377 automated DNA sequencer.

2.3 Polymerase chain reaction (PCR) and agarose gel analysis

All PCR reactions were carried out in a 25 µl final volume in sterile 0.2 ml thin-walled PCR tubes and cycled using a C1000 model Thermal Cycler (BIO-RAD). An agarose gel (1%) was prepared according to the standard protocol. DNA ladders 2-Log (New England BioLabs) and 0.5 kb (Fermentas) were run alongside the samples to enable analysis of DNA fragment sizes in the samples. Gels were viewed using an Uvitec gel documentation system.

2.4 Construction of pSAT-1 expression vector

A 1,065-bp DNA fragment corresponding to the *fba* coding sequences was amplified by PCR using genomic DNA of *N. meningitidis* strain MC58 and the primer pair FBA_E1 and FBA_E2 incorporating *Bam*H1 and *Kpn*I sites, respectively **(Table 2).** The DNA fragment was purified and ligated to the pEXPNT-TOPO expression vector (Invitrogen) according to the manufacturer's instructions. Screening for positive transformants was performed by colony PCR amplification of the desired DNA fragment, restriction digestion with *Bam*H1 and *Kpn*I and DNA sequencing. A construct containing correctly oriented *fba* was chosen and used to transform *E. coli* BL21 (DE3) pLysS according to the manufacturer's instructions. The pEXP/NT-TOPO-based constructs drive expression of the recombinant protein with N-terminal 6-histidine tag under the control of phage T7 promoter.

2.5 Construction of pSAT-9 expression vector

A DNA fragment corresponding to the *fba* coding sequence was amplified by PCR using genomic DNA of *N. meningitidis* strain MC58 and the primers FBA_pQE70 (F) and FBA_pQE70 (R) incorporating *Sph*I and *Bgl*II sites, respectively **(Table 2).** To construct pSAT-9 *Sph*I and *Bgl*II-digested, gel-purified *fba* PCR product was then ligated with pQE70 according to the manufacturer's instructions (QiagenUK). Positive clones/transformants were screened for the successful ligation of *fba* by colony PCR, restriction digestion with *Sph*I and *Bgl*II and DNA sequencing to determine in frame ligation. A clone that contained *fba* in the correct orientation for translation was selected and used to transform *E. coli* BL21 (DE3) pLysS (Invitrogen) according to the manufacturer's instructions.

2.6 Pilot expression of the recombinant FBA (rFBA)

E. coli strain BL21 (DE3) pLysS harboring either pSAT-1 or pSAT-9 expression plasmid were grown overnight in LB broth containing 100 µg ml^{-1} ampicillin. The following day, 500 µl of the overnight culture was inoculated into fresh 10 ml LB/ampicillin broth. The cultures were allowed to grow to mid log phase (OD_{600} = 0.6). The culture was split into two 5 ml culture aliquots and IPTG was added to a final concentration of 1 mM to one of the cultures aliquot. The culture tubes were then incubated at 37°C for 4 h with shaking and 500 µl aliquot was removed from each culture at times 0, 1, 2, 3, and 4 h, and cell were harvested by centrifugation at 13,000×*g*. The cell pellets were either stored at -20°C or re-suspended in 100 µl of 1× SDS-PAGE sample Buffer. The samples were heated to 100°C for 5 min and subsequently resolved by SDS-PAGE followed by western blot analysis.

2.7 Purification of rFBA under denaturing conditions

Protein purification was achieved by re-suspending the culture pellet from 20 ml culture in 1 ml lysis buffer B (100 mM NaH_2PO_4, 10 mM Tris-Cl, and 8 M Urea, pH 8.0) and sonicated (20 s pulses for 5 min on ice) using a (SoniPrep sonicater MSE 150) to disrupt the cells. The lysate from disrupted cells was centrifuged at 8,000×*g* for 30 min at 4°C. The supernatant was collected in a sterile Eppendorf tube for purification of the recombinant protein. Purification was carried out using a Ni-NTA spin kit (Qiagen, UK). Firstly, Ni-NTA spin columns were equilibrated with 600 µl of buffer B, and centrifuged at 700 × *g* for 2 min to drain the buffer and then 600 µl of supernatant collected from the lysate of disrupted cells was loaded into pre-equilibrated columns and centrifuged at 700 × *g* for 2 min. Secondly, the spin columns were washed twice with approximately 600 µl of buffer C (100 mM NaH_2PO_4, 10 mM Tris-Cl, and 8 M Urea, pH 6.3). The third step was the elution of pure proteins bound to spin column with buffer E (100 mM NaH_2PO_4, 10 mM Tris-Cl, and 8 M Urea, pH 4.5). The protein elutes were stored at -20°C

2.8 Purification of rFBA under native conditions

Briefly, the culture pellet from a 1-litre culture of *E. coli* BL21 (DE3) pLysS cells containing pSAT-9 and induced with IPTG for 3 h, was re-suspended in the lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 7.4) and the cells were then disrupted by sonication (10s on, 20s off pulses for 10 min on ice) in ice water. Soluble and insoluble fractions were separated by centrifugation (8,000 × *g* for 30 min at 4°C). Five hundred µl of Hispure™ cobalt resin (Thermo) was centrifuged at 2,000 × *g* for 1 min to drain the storage buffer and then the resin was added to the soluble fraction containing the rFBA. The supernatant-resin mixture was then incubated overnight at 4°C to allow binding of the tagged protein to the resin. The following day, the mixture was passed through gravity purification columns (SigmaUK). The flow through was collected and re-applied to the column to maximize protein binding. The resin was then extensively washed with approximately 10 column volumes of wash buffer (50 mM NaH_2PO_4, 300 mM NaCl, 15 mM imidazole, pH 7.4) to minimize the yield of non-specific proteins in the eluted fraction. The protein bound to the resin was then eluted in elution buffer (50 mM NaH_2PO_4, 300 mM NaCl, 300 mM imidazole, pH 7.4). The purity of the protein was examined by SDS-PAGE.

2.9 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

The recombinant proteins were electrophretically separated by 10% SDS-PAGE using Mini- Protean III equipment (BIO-RAD, UK) following the method of Laemmli (Laemmli, 1970). Briefly the protein samples were prepared by mixing bacterial cell pellets with 1 × SDS-PAGE sample buffer and heated at 100°C for 5 min. An aliquot of 10µl of sample was loaded into each well of the gel and 10µl of pre-stained protein markers (New England Biolabs) were loaded into an adjacent lane. Proteins separated by SDS-PAGE were visualized by staining with SimplyBlue SafeStain (Invitrogen, UK) according to the manufacturer's instructions. Gels were scanned using a GS-800 calibrated densitometer (BIO-RAD, UK).

2.10 Western blot analysis

For Western blot analysis was performed essentially as described previously (Tunio *et al.*, 2010). Briefly, the protein mixtures following separation on 10% SDS-PAGE, were transferred onto a nitrocellulose membrane (Amersham Biosciences) in semi-dry blotting buffer (5.82 g Tris base, 2.93 g Glycine, 3.75ml 10% SDS, 200 ml methanol) using a Trans-Blot SD semidry transfer cell (BIO-RAD according to manufacturers instructions.. The membranes were incubated in 1% Bovine serum albumin (BSA) (Sigma, UK) for 30 min and then probed with mouse anti-pentahistidine antibody (Qiagen, UK) or rabbit primary antibody diluted 1:10,000 and 1:1000, respectively, in blocking buffer (5% [wt/vol] non fat dry milk, 0.1% [vol/vol] Tween 20 in 1 × phosphate-buffered saline [PBS]) and incubated overnight with shaking at 4°C. The following day, the membrane was washed three times 15 min each with PBS containing 0.05% Tween-20 (PBS-Tween), and incubated into secondary antibody (anti-mouse IgG conjugated to alkaline-phosphtase or anti-rabbit conjugated to alkaline phosphtase) (Sigma,UK) at the concentration of 1:30,000 for 2h at room temperature. After 2 h the membrane was washed thrice with PBS-Tween for 15

min each time. The membrane was then developed using the BCIP (5-bromo-4-chloro-3-indolylphosphate)–Nitro Blue Tetrazolium liquid substrate (PerkinElmer™ UK). The membrane was finally washed with dH_2O and a digital image of the membrane was taken using a GS-800 calibrated densitometer (BIO-RAD, UK).

2.11 Production of a rabbit antiserum against purified recombinant FBA

Rabbit polyclonal antibodies against the denatured and non-denatured purified FBA proteins were raised in New Zealand white female rabbits. Typically, rabbits were immunized three times at 2-week intervals with 30 µg of protein emulsified in Freud's complete (first immunization) or incomplete adjuvant. After two doses, the rabbits were test bled, boosted once more, and sacrificed 10 days later.

3. RESULTS AND DSCUSSION

3.1 Cloning and expression of *fba* in pEXP/NT-TOPO

The *fba* gene consisting of 1,065 bp, (which encodes a protein of a 354 amino acids) was amplified by PCR **(Fig 1a)** and purified to ligate with the pEXP-NT/TOPO expression vector followed by transformation of *E. coli* JM109 cells for plasmid propagation and purification. The pEXP-NT/TOPO expression vector lacks the white/blue phenotype based screening system; therefore, the screening for the successful clones was initially attempted by colony PCR **(Fig 1b)**. In order to ascertain the orientation of *fba*, the plasmid DNA was sequenced. The plasmid containing *fba* in correct orientation was

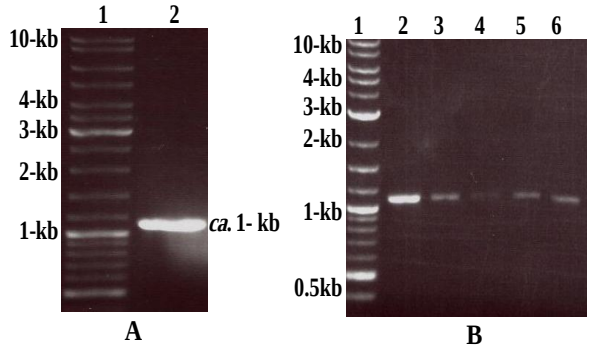


Fig. 1. Agarose gel analysis demonstrating the (A) PCR amplification of *fba*. Lane 1, DNA markers, lane 2, PCR product of *fba*. (B) colony PCR amplification of *fba*, lane 1, DNA markers, lane 2, PCR product of *fba* from MC58 chromosomal DNA (Control), lanes, 3-6, putative transformant colonies.

designated as pSAT-1 and used for expression of rFBA protein.Optimal expression of rFBA was achieved in *E. coli* BL21 (DE3) pLysS cells harboring the plasmid pSAT-1 following induction with IPTG. Expression was confirmed by SDS-PAGE followed by either staining or western blot analysis. A recombinant protein band with apparent molecular weight of *ca*. 38-kDa corresponding to the rFBA was observed in induced samples on the SDS gel after staining with SimplyBlue SafeStain **(Fig 2a)**. Western blot analysis using anti-pentahistidine antibodies also detected thetagged recombinant protein corresponding to the full length rFBA **(Fig 2b).** A faint protein band was also observed in un-induced samples. This was probably due to leaky expression of the gene without induction as it was absent in negative control cells containing the same vector without the insert.

3.2 Purification of rFBA under denaturing conditions

Due to the insoluble nature of recombinant protein expressed with N-terminal His-tag, purification was performed under denaturing conditions. The culture of *E. coli* harbouring pSAT-1 was induced with IPTG in a mid log phase and further grown for 3h. Following sonication of the harvested cells, purification of the recombinant protein was successfully achieved under denaturing conditions using affinity chromatography. The protein was eluted in elution buffer containing urea. After purification urea was removed by dialysis against PBS D-tube-dialyzer. Purified protein was analysed by SDS-PAGE to confirm the purity and integrity of the purified protein **(Fig 3a)** and western blot analysis using RαFBA **(Fig. 3b)** and anti-pentahistidine **(Fig. 3c)** recognized a strong protein band corresponding to FBA.

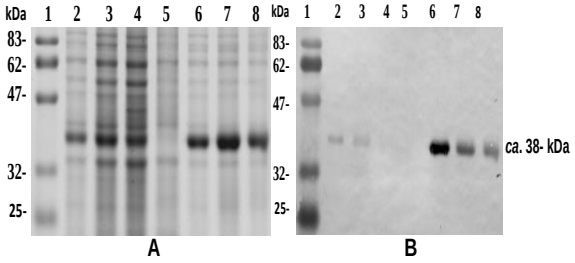


Fig. 2. SDS-PAGE and western blot analysis showing the pilot expression of FBA. (A) Lane 1,protein markers; lanes 2-4, whole cell lysate of non-induced cultures after , 1, 2, and 3 h, respectively; lane 5, lysate from *E. coli* containing empty vector, lanes 6-8 whole cell lysate of IPTG-induced cultures after 1, 2, and 3 h, respectively. (B) Western blot analysis using anti-penta histidine antibodies. Lane 1, protein markers; lane 2-4, un-induced samples lane 5, lysate from *E. coli* containing empty vector, lanes 6-8 induced samples.

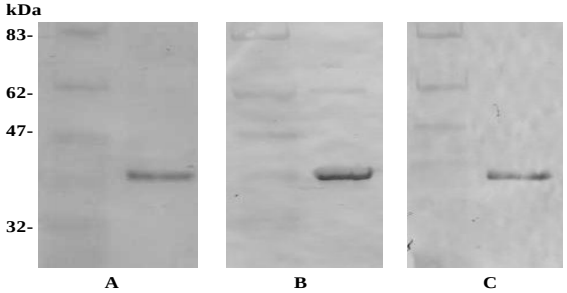


Fig. 3. SDS-PAGE analysis (A) showing the purity of the recombinant FBA purified under denaturing conditions. (B) Western blot analysis shows that recombinant FBA is recognized by RαFBA and (C) anti-pentahistidine antibodies

In order to purify the recombinant protein under native condition, the protein should be in soluble form. The solubility of the expressed rFBA was therefore determined by re-suspending the cell pellet in PBS. Following 30 min incubation at room temperature, cells were disrupted by sonication and centrifuged to separate soluble and insoluble fractions. Both fractions were collected separately and analysed by SDS-PAGE **(Fig. 4a)** followed by western blot analysis using anti-penta histidine antibodies **(Fig. 4b).** A strong protein band corresponding to FBA was detected in insoluble fraction whereas a weak band was present in soluble fraction of expressed protein suggesting that the rFBA protein was expressed largely as insoluble protein.

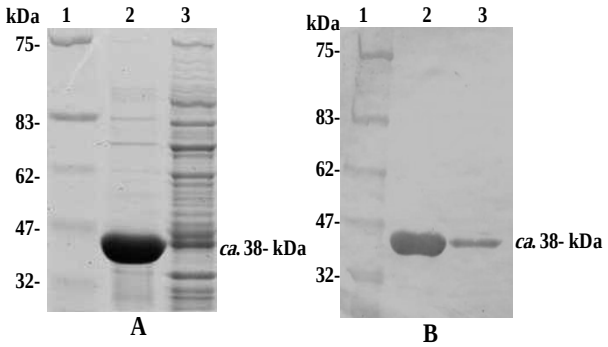


Fig. 4. SDS-PAGE and Western blot analysis showing solubility of the rFBA. (A) Protein were resolved by 10% SDS-PAGE Lane 1, protein markers; lanes 2 pellet (insoluble fraction; inclusion bodies); lane 3, supernatant fraction. (soluble fraction), (B) Westernblot analysis of protein resolved by SDS-PAGE. Lane 1, protein markers; lane 2, pellet (insoluble fraction; inclusion bodies); lane 3, supernatant fraction.

Following several unsuccessful attempts to purify N-terminally His-tagged protein under native conditions, the rFBA was purified under denaturing conditions. Failure to purify protein under native conditions may possibly be due to the insoluble form of the protein, as confirmed by finding that majority of the proteins were in the pellet (inclusion bodies) fraction rather than in supernatant of expressed samples. Despite optimisation of expression parameters such as lowering the growth temperature (20, 25, 30°C), adding less concentration of IPTG (0.5mM) at late exponential phase which is believed to improve the solubility of recombinant proteins, several attempts to express the protein in a more soluble form were unsuccessful. The reason for this insolubility is unclear; however, it might be related to the nature of the vector promoter (T7 promoter) or be intrinsic to the FBA protein. This insolubility issue was not just the case with meningococcal FBA, but this enzyme expressed with an N-terminal His-tag from *Mycobacterium tubeculosis* was also shown to be functionally inactive and insoluble protein (Ramsaywak *et al.*, 2004). In that study, to improve the yield of soluble protein efforts were made to express the tagged FBA at low temperature (15°C) and also by co-expression with different chaperones (GroES and GroEL; or DnaJ, Dnak, and grpE). These parameters although were shown to improve a little the yield of soluble protein but failed to yield an active enzyme (Ramsaywak *et al.*, 2004). Another possible explanation for inability to purify the protein under native conditions might also be due to the manner in which the protein is folded in its native form, such that the histidine-tag is buried within the protein and may not be exposed sufficiently to be purified by affinity chromatography.

3.4 Cloning of *fba* in pQE70 expression vector

The rFBA with N-terminal His-tag was expressed and successfully purified under denaturing conditions; however, to examine the functional activity of the recombinant protein and to generate polyclonal antibodies against conformational epitopes of the protein, *fba* was cloned with C-terminal 6×His-tag in an endeavour to express recombinant proteins in soluble form to facilitate subsequent purification under native conditions. The *fba* gene was amplified by PCR using primers incorporating *Sph*I and *Bgl*II restriction sites to facilitate the ligation with pQE70. The purified, digested *fba* product was successfully ligated to pQE70 after both molecules were digested with *Sph*I and *Bgl*II followed by transformation of *E. coli* JM109 cells. In order to confirm successful cloning, a number of putative recombinant clones were selected and screened for the presence of *fba* by colony PCR. Several clones produced an expected band of *ca.* 1-kb, which were selected and propagated for purification of plasmid DNA. Finally, one of the clones that were positive by PCR and restriction digestion was sequenced. The sequencing results confirmed that the ligation was in frame with the start codon of the vector and the sequences were identical to that of the *fba*. This clone was selected for expression of recombinant protein and subsequent purification under non-denaturing conditions.

3.5 Expression and purification of rFBA under native conditions

E. coli BL21 (DE3) pLysS cells harboring the plasmid pSAT-9 were used to express rFBA. Optimal expression was achieved after 3 h induction with IPTG. Expression was confirmed by SDS-polyacrylamide gel followed by western blot analysis. The Recombinant FBA was purified under native conditions from a 1-litre culture of *E. coli* harboring pSAT-FBA by affinity chromatography. Purified protein was analysed by SDS-PAGE to confirm the purity and predicted molecular weight. SDS-PAGE analysis demonstrated a strong recombinant protein band with an apparent molecular weight of *ca.* 38-kDa corresponding to the FBA.

Western blot analysis using anti-penta histidine and RαFBA antisera also detected the protein band representing rFBA (data not shown) to raise polyclonal antisera to facilitate subsequent characterisation.

4. CONCLUSION

The present study describes the cloning of the *fba* gene of *N. meningitidis* strain MC58 and, the expression and purification of recombinant FBA protein for determining its role in pathogenesis of meningococcal disease. The choice of gene for investigation in this study was based on the premise that glycolytic enzymes are often found localized to the surface of a variety of bacterial and fungal pathogens and shown to have potential roles in adhesion to various host molecules such as fibronectin (Esgleas *et al.*, 2008), laminin (Gozalbo *et al.*, 1998; Pancholi & Fischetti, 1992), plasminogen (Agarwal *et al.*, 2008), mucin (Alvarez *et al.*, 2003) and cadherin (Blau *et al.*, 2007). It was hypothesized that similar to glycolytic enzymes from other pathogens, meningococcal FBA may be a multi-functional protein, which performs additional roles unconnected to glycolysis in *N. meningitidis*. In *N. meningitidis* strain MC58 genome there is a single copy of *fba* (NMB1869) predicted to encode FBA. Sequence analysis shows that FBA is highly-conserved at the amino acid level, and is a Class-IIB enzyme.

In summary, the present study demonstrates the cloning of the FBA enzyme and subsequent expression and purification of recombinant protein either under denaturing or non-denaturing conditions. Polyclonal antisera were also raised in rabbit against native and denatured rFBA. Both antisera were shown to react against the cognate purified proteins in western blot experiments.

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