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Full Length Research Paper

Mutational analysis of fructose-1,6-bisphosphate aldolase of *Neisseria meningitidis* serogroup B

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Fructose-1,6-bisphosphate aldolase (FBA) is a classical cytoplasmic glycolytic enzyme which, despite lacking a predicted signal peptide, has been demonstrated to be expressed and transported to the surface of numerous Gram-positive bacteria and shown to interact with host molecules and perform non-glycolytic biological functions. Genome-based studies have also demonstrated that the glycolytic pathway appears to be non-functional in the meningococcus due to absence of phosphofructokinase, one of the important enzymes in this pathway. This study aimed to investigate whether the FBA, a so-called housekeeping enzyme, is required for maximal *in vitro* growth of *N. meningitidis*. An FBA knock-out mutant was created in *N. meningitidis* using an inverse polymerase chain reaction (PCR) and allelic exchange methodology. Phenotypic analysis of FBA-deficient mutant strains such as growth profiling experiments showed that the FBA-deficient mutant grew at the same rate (in broth culture and on solid media) as the wild-type strain, suggesting that FBA is not required for optimal growth of *N. meningitidis* under the *in vitro* conditions tested. No differences in either colony or bacterial cell morphology (using light microscopy) were observed. In summary, despite being a central enzyme in the glycolytic cycle, FBA is not required for *in vitro* growth of *N. meningitidis*.

Key words: *Neisseria meningitidis*, aldolase, mutagenesis, growth kinetics, glycolytic cycle.

INTRODUCTION

Neisseria meningitidis (the meningococcus) is an exclusively human nasopharyngeal commensal, which remains a leading cause of life-threatening meningitis and septicemia. *N. meningitidis* is a Gram-negative, aerobic, diplococcus, non-sporing, aflagellate, oxidase and catalase positive, may be encapsulated or un-encapsulated, and is a member of the bacterial family *Neisseriaceae* (Ala'Aldeen and Turner, 2006). *N. meningitidis* is not only a commensal of the human nasopharynx but also an important and devastating human pathogen (Schneider et al., 2007), affecting all age groups but primarily targeting children and young adults (Soriano-Gabarro et al., 2002; Harrison, 2006). Despite a huge success in the development of drugs and effective vaccines, the problem of disease due to *N. meningitidis* appears to be far from solved (Peltola, 1983) and still remains a serious threat to global health occurring sporadically throughout the world (Milonovich, 2007).

Glycolysis is the key pathway for carbohydrate degradation (glucose) in nearly all organisms and requires a cascade of nine enzymatic reactions. Most of these reactions are reversible during the gluconeogenic cycle (Fourrat et al., 2007). Glycolysis can be generally divided into two phases, the priming phase and the energy-yielding phase. The priming phase uses two moles of adenosine triphosphate (ATP) to convert glucose to fructose-1,6-bisphosphate. In the second phase, fructose-1,6-bisphosphate is further converted stepwise into pyruvate with the production of four moles of ATP and two moles of NADH (Kim and Dang, 2005). FBA catalyses the reversible cleavage of fructose-1,6-bisphosphate into two triose sugars: Dihydroxyacetone

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Table 1. Bacterial strains and plasmids.

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
N. meningitidis		
MC58	wild-type serogroup B strain	Tettelin et al. (2000)
MC58Δ*fba*	*fba* deletion and replacement with kanamycin cassette	This study
Plasmids		
pGEM-T Easy	Cloning vector	Promega
pJMK30	Source of kanamycin resistance cassette	van Vliet et al. (1998)
pSAT-2	pGEM-T Easy vector cloned with 2.3 kb insert of FBA plus flanking DNA	This study
pSAT-4	pSAT3 cloned with kanamycin resistance cassette of 1.5 kb	This study

phosphate (glycerine-P) and glyceraldehyde 3-phosphate (Wehmeier, 2001; Ramsaywak et al., 2004). In addition to its metabolic function, studies have demonstrated that FBA is present on the surface of several microbial pathogens and may facilitate their adhesion to host tissues by interacting directly with host surface ligands. For example, in *S. pneumoniae*, surface-associated FBA was shown to bind to cadherin (Blau et al., 2007) and also shown to be immunogenic in humans and capable of inducing a protective immune response against *S. pneumoniae* in mice (Ling et al., 2004). FBA was found to be a surface-localized immunogenic protein in *S. suis* (Zongfu et al., 2008), and a possible role for FBA in immunity to a nematode parasite *Onchocerca volvulus* has also been reported (McCarthy et al., 2002). Interestingly, genome-based study on meningococcal strain MC58 has revealed that glycolysis (also known as the Embden Meyerhof Parnas [EMP] pathway) is not involved in pyruvate synthesis in *N. meningitidis* due to the absence of one essential enzyme phospho-fructokinase (EC 2.7.1.11) in the pathway. The generation of pyruvate from glucose has, instead, been shown to occur through the Entner Douderoff (ED) and the Pentose Phosphate (PP) pathways, confirming that EMP (glycolysis) is non-functional in *N. meningitidis* (Baart et al., 2007). The presence of an intact gene encoding this enzyme in the meningococcal genome suggests an alternative role for the enzyme, as in the absence of such a role, spontaneous mutations would be expected to accumulate in the encoding sequence generating a pseudogene.The aim of this current study was to investigate the role of FBA by creating an *fba*-knock-out mutant in *N. meningitidis* and to determine the growth characteristics between wild-type MC58 and *fba* mutant strains.

MATERIALS AND METHODS

Bacterial strains, growth conditions and media

Bacterial strains and plasmids used in this study are listed 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 (30 μg ml^{-1}). Meningococcal strains (Table 1) (stored at -80°C in a mixture of 50% MHB and sterilized glycerol) were grown on Brain heart infusion (BHI) agar and/or Brain heart infusion broth or Muller-Hinton agar (MHA) or Muller-Hinton broth (MHB) or Dulbecco's modified eagle medium (DMEM) supplemented with Vitox (Oxoid) at the concentration suggested by the manufacturer (Oxoid) or 2% Fetal calf serum (Invitrogen) or 10% Human serum, and where appropriate, kanamycin (50 μg ml^{-1}) were used to select mutant strains and incubated in an atmosphere of 5% CO_2 at 37°C with or without shaking.

DNA manipulation

Chromosomal DNA of *N. meningitidis* was prepared by using a DNeasy blood and tissue kit (Qiagen), using the protocol for bacterial cells recommended by the manufacturer. The plasmid purification was achieved from 5 ml overnight cultures using a QIAprep spin kit (Qiagen) according to the manufacturer's instructions. Taq polymerase, restriction enzymes and T4 DNA ligase were purchased from Roche. 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). DNA sequencing was carried out at the School of Biomedical Sciences (University of Nottingham) on an ABI 377 automated DNA sequencer.

Polymerase chain reaction (PCR) and agarose gel analysis

All PCR reactions were performed in a 25 μl final volume using sterile 0.2 ml thin-walled PCR tubes and cycled using a C1000 model Thermal Cycler (BIO-RAD). An agarose gel (0.8 to 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 size in the samples. Gels were viewed using an Uvitec gel documentation system.

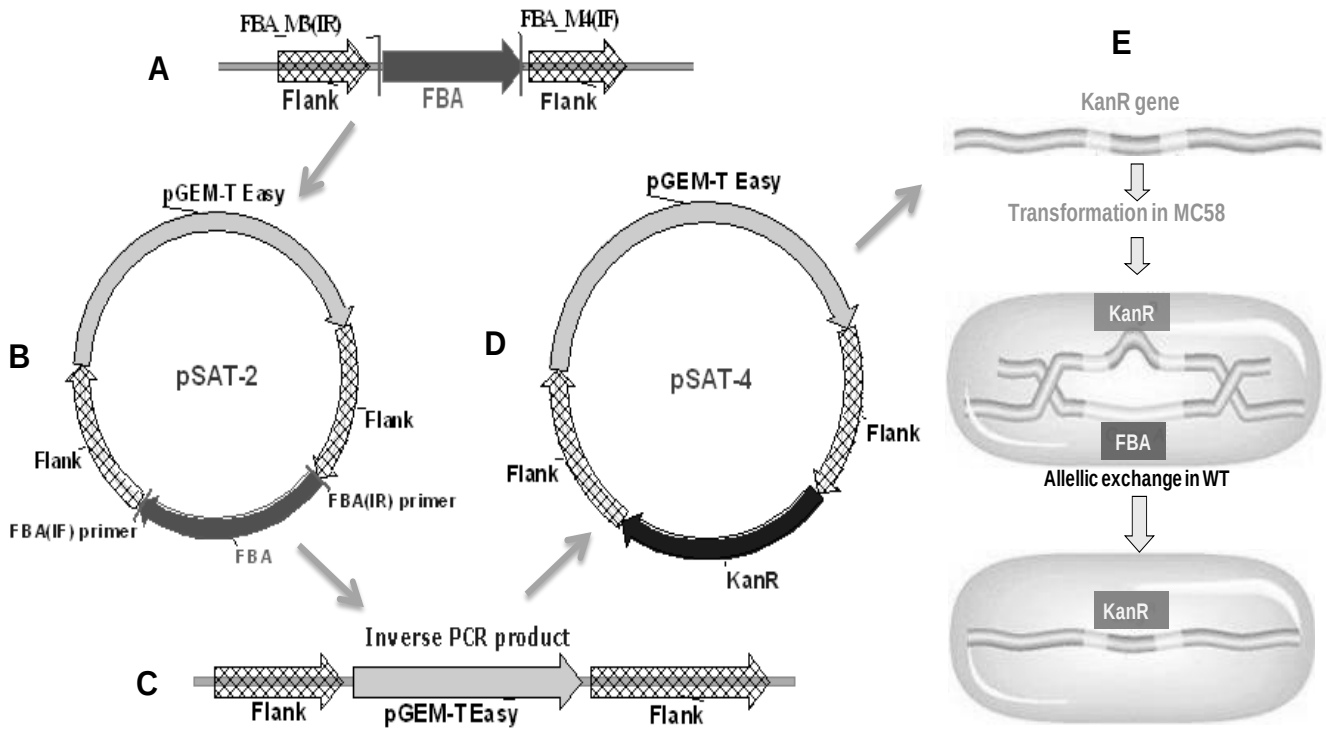

Figure 1. Schematic diagram representing the mutagenesis strategy for *fba* in *N. meningitidis* (A) A 2.3 kb DNA fragment encompassing *fba* and flanking DNA; (B) Cloning of DNA fragment, encompassing *fba* and flanking DNA sequences, in pGEM-T Easy to generate pSAT-2; (C) Inverse PCR product containing the flanking DNA and pGEM-T easy vector for ligation of Kan^R resistance marker; (D) Mutagenic plasmid designated pSAT-4 containing Kan^R and inverse PCR product;. (E) Introduction of pSAT-4 in *N meningitidis* by natural transformation to yield FBA knock-out mutant.

Engineering a mutagenic construct using *fba* sequences plus flanking DNA

A *ca.* 2.3 kb fragment of DNA (Figure 1A) consisting of the *fba* gene, 1 kb of upstream and *ca*. 300 bp of downstream flanking DNA was amplified by PCR using the primer pair FBA_M1 (F) and FBA_M2 (R) (Table 2). The PCR product was used to ligate with the pGEM-T easy vector according to the manufacturer's instructions. Recombinant clones were confirmed by restriction digestion and PCR analysis using the primers FBA_M1 (F) and FBA_M2 (R) (Table 2). The resulting plasmid was designated pSAT-2. The plasmid pSAT-2 (Figure 1B) was subjected to inverse-PCR using the primer pair FBA_M3 (IR) and FBA_M4 (IF) (Table 2), The resulting PCR product (Figure 1C) was digested with *Bgl*II to enable insertion of kanamycin resistance cassette, which was prepared from the plasmid pJMK30 (van Vliet et al., 1998) by digestion with *Bam*HI. The *Bgl*II-digested and purified i-PCR product was ligated to *Bam*HI-digested kanamycin resistance cassette. The resulting plasmid was designated pSAT-4 (Figure 1D).

Mutagenesis by natural transformation and allelic exchange in *N. meningitidis*

N. meningitidis strain MC58 cells were grown into BHI broth supplemented with Vitox and incubated overnight in 5% CO_2 at 37°C. The following day, overnight broth cultures were diluted 1:20 in fresh BHI broth and grown to an optical density of 0.2. An aliquot of 0.2 ml of the culture was transferred to a 15 ml tube with conical bottom containing 1.5 ml of BHI agar supplemented with Vitox. After incubation for 5 to 6 h at 37°C in 5% CO_2 without shaking, 10 µl (*ca.* 1 µg) of the pSAT-4 (mutagenic plasmid DNA) was added to the culture tubes and incubation was continued for 16 h. The putative transformants were selected on BHI containing kanamycin 50 µg ml^{-1}. Kanamycin-resistant *N. meningitidis* colonies were obtained and further analyzed by PCR and immunoblotting of whole cell extracts of the putative mutants.

Determining *in vitro* growth characteristics

Meningococcal growth characteristics in liquid cultures were conducted by re-suspending (1:100) overnight cultures of *N. meningitidis* in 10 ml of MH broth with and without human serum/FCS, and in DMEM with and without human serum/FCS. The following day, overnight broth cultures were diluted in fresh BHI or DMEM and adjusted to a starting OD_{600} of 0.06. The cultures were incubated with shaking at 200 rpm as described elsewhere. The OD_{600} reading was taken at hourly intervals for 8 h and then at 24 h, and experiments were performed in triplicate.

RESULTS AND DISCUSSION

Cloning of FBA plus flanking DNA

A region of DNA containing 1 kb upstream and *ca.* 300 bp downstream of the start codon of *fba* was amplified by PCR (Figure 2A). This fragment contained one copy of the neisserial DNA uptake sequence (5'-GCCGTCTGAA-3') downstream of the *fba* gene, which is required for efficient DNA uptake by natural transformation of *N. meningitidis* (Elkins et al., 1991). The amplicon was used to ligate with pGEM-T easy vector followed by

Table 2. List of primers used in this study[a].

Primer	DNA sequence[a]	Restriction site
FBA_E1(F)	CGC**GGATCC**ATGGCACTCGTATCCATGCG	*BamH*I
FBA_E2(R)	CGC**GGTACC**GTCGTCCGAACGGCGG	*Kpn*I
FBA_M1(F)	CTGCTGTGCCCGAGC	
FBA_M2(R)	CCGCTGCTGCAGGCG	
FBA_M3(IR)	GCG**AGATCT**TGTGTCTCCTTGGGCAATAGG	*Bgl*II
FBA_M4(IF)	GCG**AGATCT**GCTCCATCCAACTGGG	*Bgl*II
Kan-CTR	GACAACGCAGACCGTTCCG	
Kan-NTR	TCGCGGCCTCGAGCAAGACG	

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

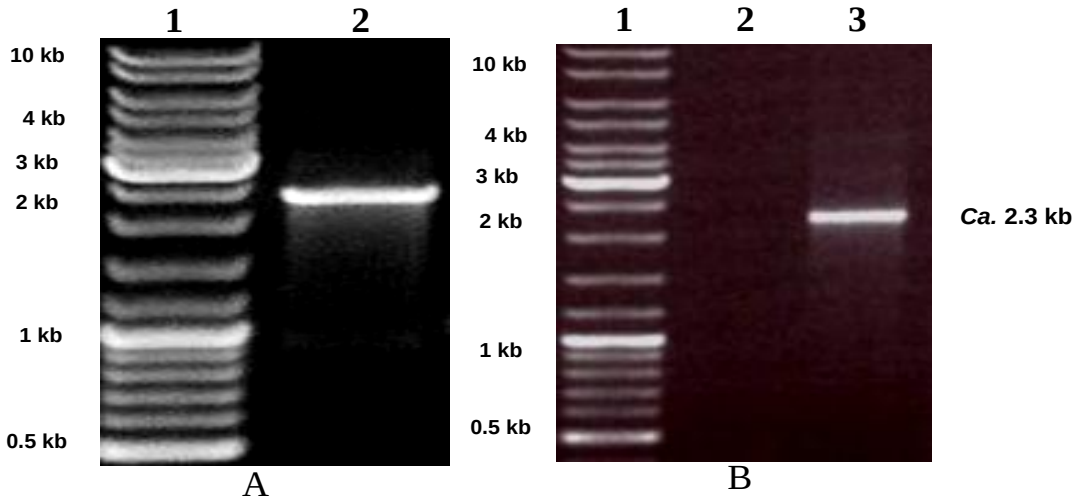

Figure 2. Agarose gel analysis (A) showing the PCR amplification of a DNA fragment consisting of *fba* and flanking DNA. Lane 1, DNA markers; lane 2, PCR product of *fba* and flanking DNA; (B) PCR amplification of DNA fragment from pSAT-2 to confirm ligation, plasmid DNA from putative transformants was used as a template for amplification of DNA fragment consisting *fba* and flanking DNA. Lane 1, DNA markers; lane 2 and 3, test samples.

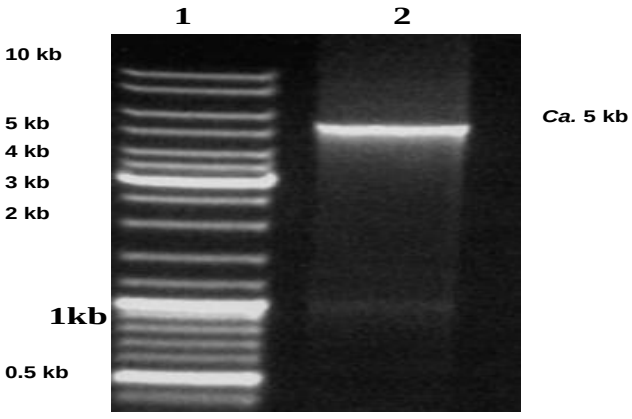

Figure 3. Agarose gel analysis (A) demonstrating the amplification of a 5 kb PCR product. The plasmid pSAT-2 was used as template DNA for inverse PCR. Lane 1, DNA markers, lane 2, inverse PCR product.

transformation of *E. coli* JM109 to yield pSAT-2. The plasmid DNA was prepared from resulting putative transformants and used to confirm the cloning of the DNA fragment (*fba* plus flanking DNA) by restriction digestion (data not shown) and PCR analysis (Figure 2B). Both approaches confirmed the successful ligation of the desired insert with pGEM-T Easy.

Mutagenesis of *fba* by inverse PCR

The plasmid pSAT-2 was used as template in inverse PCR to delete an approximately 300 bp of the *fba* gene from start codon. The PCR resulted in amplification of a 5 kb DNA fragment (Figure 3) and the introduction of a unique *Bgl*II site. The PCR product was then digested with *Bgl*II to enable the insertion of selectable marker

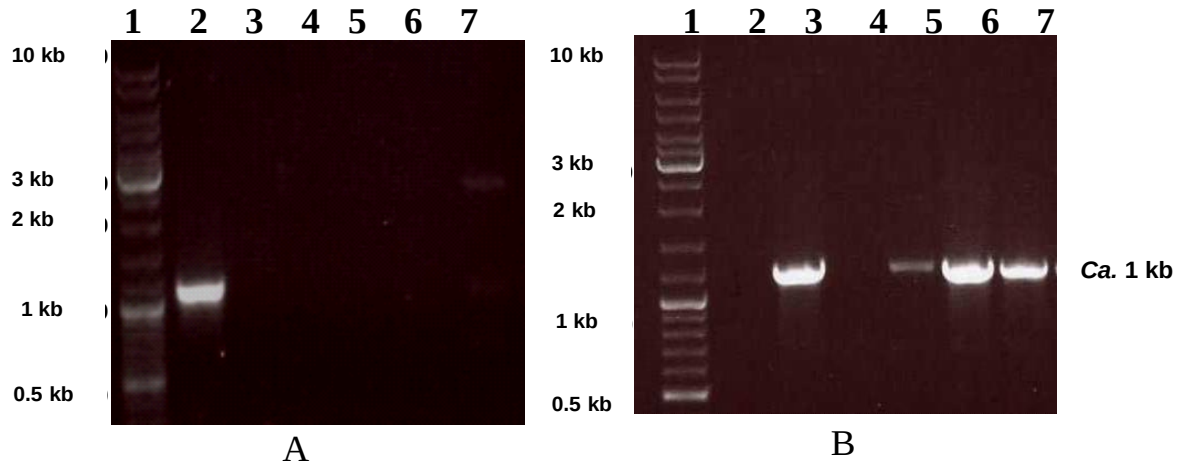

Figure 4. Agarose gel demonstrating the (A) PCR amplification of *fba* from the putative mutants. Lane 1, DNA markers; lane, 2, MC58-WT DNA; lanes 3 to 7, putative *fba* mutant DNA. (B) Orientation and presence of Kan^R cassette in putative *fba* knock-out mutants. Lane 1, DNA markers; lane 2, MC58-WT DNA (control); lanes 3 to 7, putative *fba* mutant DNA. Lane 2 failed to produce any band due to absence of the cassette in the WT, whereas a band of expected size was observed in 3, 5 to 7 lanes, confirming the successful replacement of *fba* with the Kan^R in the same orientation. Lane 4 could not produce any band suggesting that the Kan^R cassette was in opposite orientation in this mutant.

encoding resistance to kanamycin. The *Bam*HI-digested, purified antibiotic resistance cassette (encoding resistance to kanamycin) was ligated to the PCR product digested with *Bgl*II followed by transformation of *E. coli* JM109 competent cells to yield pSAT-4. The ligation of Kan^R cassette to *Bgl*II-digested PCR product was confirmed by restriction digestion and PCR analysis (data not shown). This confirmed that the Kan^R cassette was successfully cloned in the pSAT-4. The pSAT-4 was then chosen to mutate the *fba* by natural transformation in *N. meningitidis* strain MC58.

Verification of *fba* mutation in putative mutant strains

The mutation of *fba* was confirmed by the PCR analysis and immunoblotting by probing the whole cell lysate from MC58-WT and MC58Δ*fba* using RαFBA antiserum. Firstly, putative *fba* mutants were confirmed for the presence of the Kan^R cassette at proper location and orientation of the cloned cassette in the chromosome by PCR analysis using the of *fba*-specific primer pair FBA_E1 (F) and FBA_E2 (R) (Table 2) to amplify *fba* from the chromosomal DNA of the putative mutant clones. Chromosomal DNA of *N. meningitidis* strain MC58 was also amplified with the same primer pair as a positive control. The PCR resulted in amplification of an expected *ca.* 1 kb band from chromosomal DNA of the MC58-WT strain whereas as expected, no band was observed in the mutant sample (Figure 4A), indicating the successful mutation of *fba,* the mutant strain was designated as MC58Δ*fba.* To determine the orientation of the Kan^R cassette another PCR using the primer pair Kan-CTR and FBA_M2 (R) (Table 2) was performed. This PCR was expected to produce a 1 kb product where the orientation of the Kan^R cassette was the same as the deleted *fba*. This confirmation was important in order to minimize the impact of the mutation on the downstream genes (if the Kan^R was cloned in same orientation) by facilitating expression of downstream genes in the same operon. An expected 1 kb band was observed from various putative mutants (Figure 4B) confirming the presence and the correct orientation of the cloned selectable marker in mutant strains. Taken together, these results appear to have genotypically confirmed that *fba* has been substituted in putative mutant genome with Kan^R antibiotic cassette.

To further confirm that the FBA gene is not expressed in the isogenic-null mutant strain, whole cell lysate of MC58-WT and MC58Δ*fba* were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) followed by immunoblot analysis by probing the membranes with RαFBA antiserum. A strongly reactive protein band with an apparent molecular weight of *ca.* 38 kDa was detected in the lysate of the MC58-WT sample which was absent from the lysate of the MC58Δ*fba* extract (Figure 5). This demonstrated that the expression of *fba* has been abolished by the mutation. The RαFBA also detected another protein band of higher molecular weight than FBA from the lysate of both MC58-WT and MC58Δ*fba*, which was presumably non-specific band due to the polyclonal nature of antiserum. In *N. meningitidis* strain MC58, *fba* is the first of four genes that could be part of an operon, and thus, polar effects may account for

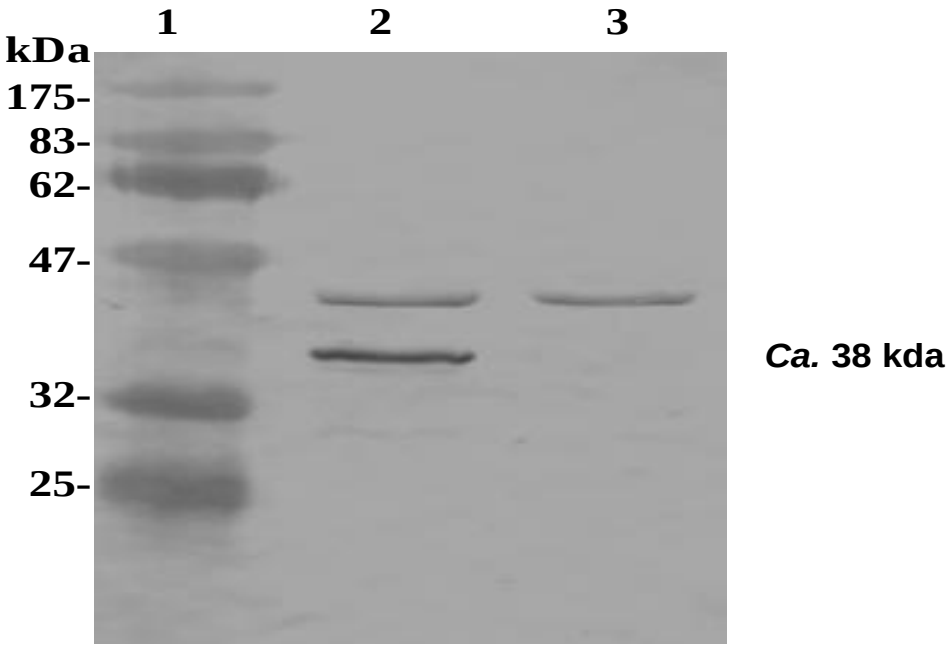

Figure 5. Immunoblot analysis demonstrating the successful deletion of *fba* in putative mutants; Lane 1, Protein markers; lane 2, whole cell extracts from MC58-WT; and lane 3, whole cell extracts from MC58 *fba* mutant.

the observed phenotypic characteristics of mutation of *fba*. To exclude this possibility, prior to undertaking any phenotypic characterization, we determined the expression of the immediately down-stream gene which encodes Factor-H binding protein (fHbp) using immunoblot analysis. The expression of fHbp was similar in both wild-type MC58 and *fba* mutant strains suggesting that the *fba* mutation has no effect on the downstream genes and thus no polar effect on adjacent genes in operon.

Determining the growth kinetics of meningococcal strains

Before undertaking phenotypic analysis of the *fba* mutant, it was first essential to demonstrate that mutation of the gene did not significantly affect the growth rate of *N. meningitidis*. Growth and colonial characteristics of mutant strains were assessed by visual inspection of colony morphology on agar plates and monitoring the rate of growth in liquid culture. The mutant strain demonstrated similar colony morphology and colour to that of wild-type strain MC58 (data not shown). The wild-type MC58, isogenic *fba* null mutant were then grown in BHI broth or DMEM with a starting OD_{600} adjusted to 0.06. Growth rate was assessed by measurement of OD_{600} of samples that were removed from the culture at hourly intervals. The growth rate of the strain with deletion of *fba* was not substantially different to that observed for wild-type MC58. Both strains were observed to grow to a similar OD_{600} by the final time point of 8 and 24 h. These experiments were performed in triplicate. In order to further explore whether the addition of normal human serum may have any effect on the growth of wild-type and mutant strains, meningococcal growth was monitored in BHI or DMEM supplemented with 10% human serum (Figure 6a). Again, no substantial differences between the growth rates of wild-type MC58 and *fba* mutant were observed (Figure 6a). These findings are consistent with the findings of Baart et al. (2007) who showed that the glycolytic pathway was non-functional in the meningococcus due to absence of one of the important enzymes in this pathway: phosphofructokinase.

Conclusion

This report explores the role of an essential glycolytic (so called housekeeping) enzyme FBA in the *in vitro* growth and pathogenesis of *N. meningitidis*. The conversion of glucose into pyruvate is accomplished in nine enzymatic reactions and most of these reactions are reversible during the gluconeogenic cycle (Fourrat et al., 2007). Recent genome-based studies have revealed that one of the glycolytic (EMP) pathway enzymes, namely phosphofructokinase (EC 2.7.1.11) is absent from *N. meningitidis* strain MC85, and also that pyruvate synthesis in *N. meningitidis* does not take place through glycolysis. These both recent findings suggest that the

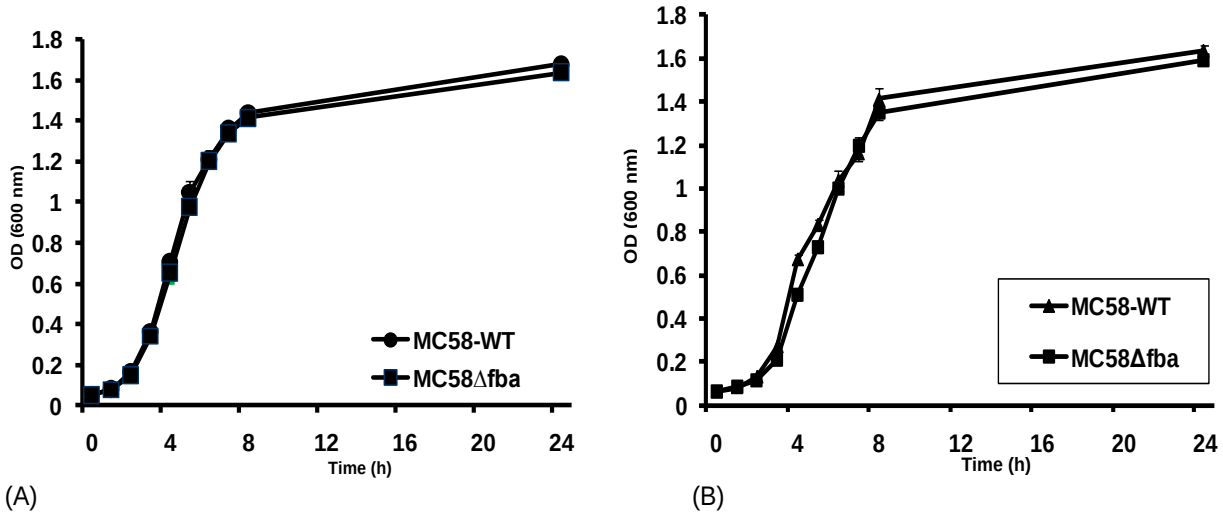

Figure 6. Growth characteristics of meningococcal mutant strains compared to their wild-type parent strain. (A) *N. meningitidis* strain MC58-WT, MC58Δ*fba*, grown in BHI broth with 10% Human serum (B) *N. meningitidis* strain MC58-WT, MC58Δ*fba*, grown in DMEM supplemented with 10% human serum.

EMP pathway in *N. meningitidis* is non-functional. The catabolism of the glucose has instead been shown to be carried out through Entner Douderoff (ED) and the Pentose Phosphate pathway (PP) (Baart et al., 2007). We undertook to investigate whether the FBA, a classical cytoplasmic enzyme, is involved in glycolytic cycle in *N. meningitidis* and also plays additional roles unconnected to glycolysis. An *fba* isogenic mutant was generated in *N. meningitidis* strain MC58 and used to investigate the growth profile of MC58 wild type and MC58Δ*fba*. The growth pattern between mutants demonstrated no significant differences, which suggests that the product of the *fba* gene was not required for optimal *in vitro* growth of *N. meningitidis* and thus has no contribution to the glycolysis in this organism. However, despite the inability to utilise the glycolysis pathway, the enzymes required for glycolysis are maintained in the meningococcal genome, presumably for other roles. In the absence of alternative roles for these enzymes, spontaneous mutations would be expected to accumulate in the encoding sequences.

REFERENCES

Ala'Aldeen DA, Turner DPJ (2006). Neisseria meningitidis. In Principles and Practice of Clinical Bacteriology (Second Edition). Stephen H, Gillespie PMH (ed), pp.205-220.

Baart G, Zomer B, de Haan A, van der Pol L, Beuvery EC, Tramper J, Martens D (2007). Modeling Neisseria meningitidis metabolism: from genome to metabolic fluxes. Genome Biol. 8: R136.

Blau K, Portnoi M, Shagan M, Kaganovich A, Rom S, Kafka D, Chalifa Caspi V, Porgador A, Givon-Lavi N, Gershoni JM, Dagan R, Mizrachi Nebenzahl Y (2007). Flamingo cadherin: a putative host receptor for *Streptococcus pneumoniae*. J. Infect. Dis. 195:1828-1837.

Elkins C, Thomas CE, Seifert HS, Sparling PF (1991). Species-specific uptake of DNA by gonococci is mediated by a 10-base-pair sequence. J. Bacteriol. 173:3911-3913.

Fourrat L, Iddar A, Valverde F, Serrano A, Soukri A (2007). Cloning, gene expression and characterization of a novel bacterial NAD-dependent non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase from Neisseria meningitidis strain Z2491. Mol. Cell. Biochem. 305(1-2):209-219.

Harrison LH (2006). Prospects for Vaccine Prevention of Meningococcal Infection. Clin. Microbiol. Rev. 19:142-164.

Kim JW, Dang CV (2005). Multifaceted roles of glycolytic enzymes. Trends Biochem. Sci. 30:142-150.

Ling E, Feldman G, Portnoi M, Dagan R, Overweg K, Mulholland F, Chalifa-Caspi V, Wells J, Mizrachi-Nebenzahl Y (2004). Glycolytic enzymes associated with the cell surface of *Streptococcus pneumoniae* are antigenic in humans and elicit protective immune responses in the mouse. Clin. Exp. Immunol. 138:290-298.

McCarthy JS, Wieseman M, Tropea J, Kaslow D, Abraham D, Lustigman, S, Tuan R, Guderian RH, Nutman TB (2002). *Onchocerca volvulus* glycolytic enzyme Fructose-1,6-Bisphosphate Aldolase as a target for a protective immune response in humans. Infect. Immun. 70:851-858.

Milonovich LM (2007). Meningococcemia: Epidemiology, Pathophysiology and Management. J. Pediatric Health Care, 21: 75-80.

Peltola H (1983). Meningococcal disease: still with us. Rev. Infect. Dis. 5:71-91.

Ramsaywak PC, Labbe G, Siemann S, Dmitrienko GI, Guillemette JG (2004). Molecular cloning, expression, purification, and characterization of fructose 1,6-bisphosphate aldolase from Mycobacterium tuberculosis-a novel Class II A tetramer. Protein Expression Purif. 37:220-228.

Schneider MC, Exley RM, Ram S, Sim RB, Tang CM (2007). Interactions between Neisseria meningitidis and the complement system. Trends Microbiol. 15:233-240.

Soriano-Gabarro M, Stuart JM, Rosenstein NE (2002). Vaccines for the prevention of meningococcal disease in children. Semin. Pediatr. Infect. Dis. 13:182-189.

Tettelin H, Saunders NJ, Heidelberg J, Jeffries AC, Nelson KE, Eisen JA, Ketchum KA, Hood DW, Peden JF, Dodson RJ, Nelson WC, Gwinn ML, DeBoy R, Peterson JD, Hickey EK, Haft DH, Salzberg SL, White O, Fleischmann RD, Dougherty BA, Mason T, Ciecko A, Parksey DS, Blair E, Cittone H, Clark EB, Cotton MD, Utterback TR, Khouri H, Qin H, Vamathevan J, Gill J, Scarlato V, Masignani V, Pizza M, Grandi G, Sun L, Smith HO, Fraser CM, Moxon ER, Rappuoli R, Venter JC (2000). Complete Genome Sequence of

Neisseria meningitidis Serogroup B Strain MC58. Science 287:1809-1815.
van Vliet AH, Wooldridge KG, Ketley JM (1998). Iron-responsive gene regulation in a *Campylobacter jejuni fur* mutant. J Bacteriol. 180:5291-5298.
Wehmeier UF (2001). Molecular cloning, nucleotide sequence and structural analysis of the Streptomyces galbus DSM40480 fda gene: the S. galbus fructose-1,6-bisphosphate aldolase is a member of the class II aldolases. FEMS Microbiol. Lett. 197:53-58.
Zongfu W, Wei Z, Chengping L (2008). Immunoproteomic assay of surface proteins of *Streptococcus suis* serotype 9. FEMS Immunol. Med. Microbiol. 53:52-59.