12-2010

The DedA Protein Family in Selected Bacterial Species

James Cox

The DedA Protein Family in Selected Bacterial Species

By

James Cox

Undergraduate honors thesis under the direction of

Dr. William Doerrler

Department of Biological Sciences

Submitted to the LSU Honors College in partial fulfillment of

The Upper Division Honors Program

December 2010

Louisiana State University

& Agricultural & Mechanical College

Baton Rouge, Louisiana

Table of Contents

Abstract

Members of the *dedA* gene family encode bacterial membrane proteins that are largely uncharacterized but may play important roles in cell division and other cellular functions. While members of the *dedA* gene family are found in a large number of diverse bacteria, the number of homologs among species varies greatly. For instance, the *Escherichia coli* K12 genome contains eight *dedA* homologs while *Borrelia burgdorferi* B31 contains only one *dedA* homolog. As for other species studied here, *Sinorhizobium meliloti* 1021 and *Vibrio vulnificus* YJ016 contain two *dedA* homologs each.

While functions of many *dedA* family members have not been explored, simultaneous deletion of two *E. coli* dedA family genes (*yqjA* and *yghB*), encoding membrane proteins with 61% amino acid identity, results in a strain that is not viable at elevated temperatures and displays defects in cell division, protein secretion, lipid synthesis and motility at all growth temperatures (Thompkins et al., 2008). This strain is called BC202 (Δ*yqjA*::tet^r; Δ*yghB*::kan^r). These observations with BC202 suggest important roles for this conserved gene family. We were therefore interested in investigating the functions of the lone *dedA* homolog found in the genome of the Lyme disease pathogen *Borrelia burgdorferi*, annotated *bb0250*.

Expression of a cloned copy of *bb0250* in BC202 results in correction of all phenotypes although BB0250 only shares 19% amino acid identity with YqjA (and less with YghB). This suggests that the proteins have conserved functions across the wide phylogenetic gap that exists between *E. coli* and *B. burgdorferi*. BB0250 also correctly localizes to the inner membrane when expressed in *E. coli*. In this work, we have characterized the functions of these members of the dedA gene family.

Introduction

Members of the DedA protein family are found in most bacteria as well as certain members of the domains Archaea and Eukarya. While some bacteria may contain only one *dedA* homolog, other species contain multiple *dedA* homologs (Table 1). Previous research has shown that the presence of multiple *dedA* homologs can involve redundancy in protein function (Thompkins et al., 2008). Because of this protein family's widespread presence, it is an important topic of research in order to gain a better understanding of species homology. Furthermore, its role in proper cell division and membrane viability is of great interest to scientists. However, relatively few bacterial species have been chosen for scientific studies of DedA protein homology.

Escherichia coli K12 contains eight *dedA* genes: *yqjA*, *yghB*, *yabI*, *yohD*, *dedA*, *ydjX*, *ydjZ*, and *yqaA*. Of these eight homologs, *yqjA* and *yghB* have been best studied and found to have redundant functions. YghB and YqjA have 61 % amino acid identity. The other six *E. coli* homologs display roughly 25-30% amino acid identity with each other and YghB/YqjA (Liang et al., 2010).

Borrelia burgdorferi is a gram-negative spirochete that causes Lyme disease. Lyme disease is spread by the bite of infected ticks of the genus *Ixodes* and is the most common tick-borne disease in the Northern Hemisphere. Early symptoms include rash at the site of the bite (erythema migrans), fever, and headache. If left untreated, the organism can disseminate within the body and infect the heart, joints, and central nervous system causing symptoms at these diverse locations within the body. The organism can infect both mammals and arthropod vectors and has developed mechanisms involving unique patterns of gene transcription to survive in both hosts (Hovius et al. 2007).

While *Escherichia coli* K12 contains eight *dedA* genes, *Borrelia burgdorferi* contains only one homolog, potentially making it a more useful tool because of its lack of multiple *dedA* genes with possible redundant functions.

In order to achieve a better understanding of the functions of DedA homologs in various bacterial species, *Sinorhizobium meliloti* was chosen for analysis of its *dedA* genes. *Sinorhizobium meliloti* is a nitrogen fixing bacterium that is known for its symbiotic relationship with legumes. *Sinorhizobium meliloti* is capable of entering through the root hairs of a legume and traveling into plant cells where nitrogen fixation will occur. Its ability to fix nitrogen is the basis for its symbiotic relationship with such plants as legumes. By studying this bacterium's DedA homologs, further conclusions can be made not only about the entire DedA protein family, but also possibly about the intricacies of this symbiotic relationship.

Finally, *Vibrio vulnificus*, a known pathogen, was chosen based on its two *dedA* genes. This pathogen is known to cause cellulitis and septicemia in humans. Because of its pathogenic nature, this bacterium is an important topic of research due to the implications research may have on public health.

Materials and Methods

Cloning of *bb0250:* PCR amplification was performed on *Borrelia burgdorferi* B31 genomic DNA. Along with genomic DNA, forward primer 250F and reverse primer 250R (Table 2), dNTP's, 5X HF buffer, and Phusion polymerase were added. The following conditions were used: initial denaturation at 98°C for 30 seconds; 30 cycles involving denaturation at 98°C for 10 seconds, primer annealing at 55°C for 30 seconds, and elongation at 72°C for 30 seconds; a final extension at 72°C lasted 10 minutes. The amplified DNA was purified using the Qiagen QIAquick kit (Qiagen, Valencia CA). After digestion with BamHI and XbaI, this sequence was ligated into a similarly digested pET28 vector. After amplification using primers 250GF and 250GR (Table 2), the sequence was digested with XbaI and XhoI and ligated into a similarly digested pTB28 vector (Bernhardt and DeBoer, 2003). This final product contained bb*0250* in frame with an N-terminal hexahistidine tag and C-terminal Green Fluorescent Protein (GFP).

Western Blotting

This prepared pBB0250-GFP was transformed into an *Escherichia coli* strain BC202 (Δ*yghB::kan*^{R}, Δ*yqjA::tet*^{R}) (Thompkins et al., 2008). Cells were lysed and total membranes were prepared and inner and outer membranes were separated in a 30-60% sucrose gradient (Doerrler et al., 2004). The denser area represented the outer membrane while the less dense area represented the inner membrane. Twenty fractions of equal volume were then extracted. After adding SDS and loading all fractions onto a 12% SDS-PAGE gel, the samples were run at 150 volts for 1 hour. The gel was placed in 1X transfer buffer + 20% MeOH. It was then placed on a filter paper saturated in this same solution. It was covered with a piece of PVDF and a second filter paper saturated with

1X transfer buffer + SDS. This was run at 20 volts for 20 minutes in order to transfer onto the PVDF. This PVDF was then shaken in blocking buffer (1x PBS, 0.05% Tween-20, 5% nonfat dry milk) overnight. It was then shaken in the OmpA and GFP antibodies separately for 1 hour. Incubations were followed by three 5-minute washes with blocking buffer. Secondary antibody was HRP conjugated goat anti-mouse IgG (Thermo Scientific) and detection was performed with the Immun-Star kit (Bio-Rad Laboratories).

NADH Oxidase Assay

This assay tested for the presence of NADH oxidase in the inner membrane. By adding NADH, NADH oxidase presence can be confirmed. After adding NADH to each fraction from the sucrose gradient, NADH oxidase will oxidize this NADH into NAD^+. Thus, the absorbance at 340 nm of these fractions containing NADH oxidase will decrease. After taking an absorbance of each fraction, a printout displaying the absorbance of each fraction was used to create a line graph depicting NADH oxidase activity vs. fraction.

***Borrelia burgdorferi* FtsA/FtsZ**

In order to study gene expression, the *Borrelia flaB* promoter was chosen due to its constitutive expression (Guatam et al., 2009). The *Borrelia burgdorferi* plasmid pBBE22 was transformed into XLI Blue cells and plated onto kanamycin plates. Liquid cultures of these colonies were purified with the Qiagen mini-prep method to obtain purified DNA. This DNA was digested with BamHI and XbaI. The DNA was again purified and Antarctic Phosphatase was added in order to remove the 5'OH to prevent recircularization of the plasmid.

Using *flaB* primers P1F and P1R (Table 2), PCR amplification was done on *Borrelia burgdorferi* genomic DNA. This amplified DNA was digested with BamHI and XbaI and inserted into the pBBE22 plasmid with similar restriction sites. After transformation into XLI Blue cells, the cells were plated onto kanamycin plates. Next, the pBBE22-flaB plasmid was digested with XbaI and SphI.

Using primers P2F and P2R (Table 2), *Borrelia burgdorferi* FtsA/FtsZ was amplified. This sequence was digested with XbaI and XhoI before being ligated into a similarly digested pTB28 vector. The result was the FtsA protein as well as FtsZ fused to the Green Fluorescent Protein. This pTB28-FtsAZ-GFP vector was digested with XbaI and SphI. This sample was run on a 1% agarose gel and the smaller band of ~3000 bp was cut out and purified. This insert, FtsAZ-GFP, was then ligated into the previously described pBBE22-flaB plasmid at the XbaI and SphI restriction sites. This plasmid was then transformed into XLI Blue competent cells and plated onto kanamycin plates.

Meanwhile, the pTB28-FtsAZ-GFP plasmid was transformed into *E. coli* W3110 wild-type competent cells. Subsequently, a Western Blot of these cells was performed using the technique described previously along with detection via an anti-GFP antibody.

Purification of *Sinorhizobium meliloti* 1021 genomic DNA

In order to obtain *Sinorhizobium meliloti* 1021 genomic DNA, the Invitrogen Easy DNA Kit was used on an overnight culture (Invitrogen, Carlsbad CA). .5ml of cell culture was resuspended in 200µl 1X PBS. 350µl of Solution A was added and vortexed in order to lyse the cells and release DNA. The tube incubated at 65°C for 10 minutes. 150µl of Solution B was added and vortexed to precipitate the DNA. 500µl of chloroform was added and the tube was vortexed then centrifuged at 4°C for 15 minutes.

The upper phase was removed and added to 100% ethanol at -20°C. The tube was vortexed and placed on ice for 30 minutes. After a 15-minute centrifugation at 4°C, 500µl of 80% ethanol at -20°C was added and the tube was inverted 5 times in order to fully mix the solution. After a 5-minute centrifugation at 4°C, the supernatant was removed. After an additional 3-minute centrifugation, any remaining ethanol was removed. The pellet air-dried for 5 minutes before being resuspended in 100µl TE buffer. After the addition of 2µl RNase and a 30-minute incubation at 37°C, the DNA was ready for experimentation.

Amplification and Modification of *S. meliloti* 1021 genomic DNA

PCR was performed on a 1:100 dilution of the *S. meliloti* 1021 genomic DNA using primers P3F and P3R (Table 2) in conditions described previously. However, in order to obtain a more favorable environment, the 5X GC buffer was used in place of the 5X HF buffer. This buffer is made for organisms containing a high GC genetic content such as *Sinorhizobium meliloti* 1021, which has a GC content of 63%.

After purifying this PCR product with the Qiagen QIAquick PCR purification kit, it was digested with BamHI and NdeI. This sequence was then ligated into a pET28 vector that was previously digested with these same restriction enzymes. After transforming into XLI Blue cells, the cells were plated onto kanamycin plates.

Meanwhile, the amplified *dedA* homolog of *Sinorhizobium meliloti* 1021 was ligated into the vector pWSK29. This resulting plasmid, labeled pJC2, was transformed into BC202 cells and plated onto ampicillin plates.

Amplification and Modification of *Vibrio vulnificus* YJ016

Vibrio vulnificus YJ016 DNA was amplified with PCR using primers P4F and P4R (Table 2). After DNA purification with the Qiagen QIAquick PCR purification kit, the DNA was digested with BamHI and NdeI. Next, this DNA segment was ligated into a pET28 vector containing BamHI and NdeI sticky ends. Finally, the DNA was transformed into XLI Blue competent cells and plated onto kanamycin plates.

A subsequent PCR reaction was done with new primers P5F and P5R (Table 2) and the DNA was similarly purified. This time, the DNA was digested with XbaI and XhoI, and then ligated into a pTB28 vector containing these same restriction sites. Finally, this plasmid was transformed into XLI Blue cells and plated onto ampicillin plates.

Results

***B. burgdorferi* BB0250 can substitute for YqjA and YghB in *E. coli*.**

BB0250 of *Borrelia burgdorferi* shares only 19% identity and 56% similarity with *E. coli* YqjA (Figure 1A). Normally, BC202 is unable to grow at 42°C. However, after transformation of pBB0250-GFP into BC202, growth of BC202 was restored at 42°C (Figure 1B). In addition, when pBB0250-GFP (Table 3) was transformed into BC202, cell division defects that are normally observed (Figure 2B, left panel) were corrected (Figure 2B, right panel). These results suggest conservation of key functions between YqjA/YghB of *E. coli* and BB0250 of *B. burgdorferi.*

BB0250-GFP localizes to the membrane fraction of *E. coli* (Figure 2A, lanes 3 and 4). Some presumed degradation products corresponding to the size of GFP can be

found in the cytoplasm (Figure 2A, lane 3) and this likely is responsible for the fluorescence that is seen throughout these cells in micrograph images (Figure 2B).

BB0250-GFP was localized to the inner membrane in E. coli using a 30-60% isopycnic sucrose gradient to separate inner from outer membranes. Protein determination of individual fractions shows three peaks with inner membrane NADH oxidase activity centered around fraction 14 (Figure 3A) and outer membrane OmpA centered around fraction 6 (Figure 3B, top panel). BB0250-GFP colocalizes with NADH oxidase activity near fraction 14 demonstrating that the protein localizes to the inner membrane when expressed in *E. coli* (Figure 3B, bottom panel). These data show conclusively that the fusion protein is correctly targeted to the inner membrane and restores normal cell division and demonstrate that BB0250 can functionally substitute for the absence of YghB and YqjA in *E. coli* mutant BC202, thus confirming that it is a member of the *dedA* family. While membrane localization of BB0250 was not performed in *Borrelia burgdorferi* cells, it is hypothesized that BB0250 will localize to the inner membrane as it did in *E. coli* cells.

***Borrelia burgdorferi* FtsA/FtsZ**

BB0250 is an essential gene in *B. burgdorferi* (Liang et al. 2010) and loss of expression of BB0250 protein from an inducible plasmid results in marked defects in cell division. As a first step in understanding the nature of the cell division defect in the borrelia Δ*bb0250* mutant (a strain called DXL-01), we would like to localize the *Borrelia* FtsZ protein in *Borrelia* using an FtsZ-GFP fusion protein in mutant and wild type *Borrelia*. This approach has proven useful in analyzing the cell division defect of BC202 (Sikdar et al, 2010).

Attempts to insert *ftsA* and *ftsZ* between a *flaB* promoter and a Green Fluorescent Protein in the pBBE22 plasmid were successful. Additionally, transformants of XLI Blue cells containing this plasmid were obtained on kanamycin plates. The Western Blot of *E. coli* cells expressing pTB28-FtsAZ-GFP showed increased expression of FtsZ-GFP in increasing levels of IPTG (Figure 4A). These results reveal that the IPTG is inducing the synthesis of FtsZ-GFP fusion protein via the *lac* promoter. Furthermore, because of the production of FtsA, FtsZ did not cause cell toxicity due to elevated FtsZ levels. In addition, fluorescent microscopy of these cells clearly shows expression of GFP (Figure 4B). Performing a similar Western Blot on *Borrelia burgdorferi* cells will hopefully reveal similar results. In these cells, FtsA and FtsZ should be produced in the proper ratio, thus preventing cell toxicity. Furthermore, it is important to confirm the localization of FtsZ-GFP to the inner membrane. Finally, fluorescent microscopy of these cells will visually display the cellular location of FtsZ-GFP.

***Sinorhizobium meliloti* 1021**

The *Sinorhizobium meliloti* 1021 genome encodes a DedA family protein with 22% amino acid identity to E. coli YdjX (Figure 5A) with six predicted membrane spanning domains (Figure 5C). After experimenting with various environmental conditions, the PCR amplification of the *Sinorhizobium meliloti* 1021 gene was finally successful (Figure 5B). However, despite frequent attempts to ligate into a pET28 vector and transform into XLI Blue cells, no viable transformants were attained.

Nevertheless, transformation of pJC2 into BC202 (Figure 6A) revealed interesting results. While growth at 42°C was not restored to BC202, colonies of BC202 containing

pJC2 appeared much larger and had a mucoid appearance when compared to BC202 containing pWSK29 without the *Sinorhizobium meliloti* 1021 *dedA* insertion (Figure 6B).

***Vibrio vulnificus* YJ016**

The *Vibrio vulnificus* YJ016 genome encodes a DedA family protein with 32% amino acid identity to E. coli YqjA (Figure 7A) with five predicted membrane spanning domains (Figure 7C). While both PCR reactions were successful based on analysis via gel electrophoresis (Figure 7B), all attempts to successfully ligate into a vector and transform into XLI Blue cells were unsuccessful.

Discussion

Determining the necessity of the *dedA* gene to a cell as well as its ability to revert mutated cells back to wild-type phenotype is a major breakthrough. Using the *dedA* gene of one organism to replace the mutated gene in a different organism reveals an important relationship between the genes. First, because the wild-type phenotype is restored, the two genes must have similar gene products. Furthermore, this gene must be highly conserved. Finally, if this gene is highly conserved, it is clear that it plays an extremely pivotal role in cell function. In turn, understanding as much about cellular efficiency is an important step in truly understanding bacteria.

Based on restoration of wild-type phenotype to BC202 subsequent to BB0250 expression, it can be concluded that BB0250 shares functional similarity to either of the *E. coli* redundant YqjA or YghB despite BB0250 only having 19% identity and 56% similarity to *E. coli* YqjA and 19% identity and 50% similarity to *E. coli* YghB (Liang et al., 2010).

The examination of FtsA/FtsZ production in *Borrelia burgdorferi* is sure to be the topic of published articles in the future. As stated previously, a Western Blot of *Borrelia* cells containing the consecutive *ftsA* and *ftsZ* genes is important in understanding protein production as well as membrane association.

Despite success in amplifying DNA from both *Sinorhizobium meliloti* 1021 and *Vibrio vulnificus* YJ016, attempts to excise the *dedA* gene and ligate into a pET28 were unsuccessful despite numerous experimental alterations. Nevertheless, BC202 transformed with pJC2 yielded intriguing results. While SMc01718 failed to restore growth to BC202 at 42°C, the morphological changes observed at 30°C in BC202 transformed with pJC2 are fascinating. Future studies on the cause of these morphological changes will lead to an overall better understanding of SMc01718. It is important that further experiments of *Sinorhizobium meliloti* and *Vibrio natriegens* be performed. In order to fully characterize the DedA protein family, as many species as possible should be studied. While such experiments will be neither straightforward nor speedy, the conclusions could greatly influence the overall understanding of the DedA protein family.

Acknowledgements

I would first like to thank Dr. Doerrler for being so generous with his time and patience. I am extremely grateful for both. Next, thank you to Dr. Gregg Pettis and Dr. Fang Ting Liang for serving on my thesis committee. Your insight is much appreciated. Thank you to the members of the Doerrler Lab for helping me along and encouraging me. Finally,

thanks to my family and friends for encouraging me on this long road. Your support did not go unnoticed.

Literature Cited

Beck, S., V. Marlow, K. Woodall, W. Doerrler, E. James, and G. Ferguson. 2008. The *Sinorhizobium meliloti* MsbA2 protein is essential for the legume symbiosis. Microbiol. **154**: 1258-1270.

Bernhardt, T. G., and P. A. de Boer. 2003. The Escherichia coli amidase AmiC is a periplasmic septal ring component exported via the twin-arginine transport pathway. Mol Microbiol 48:1171-82.

Dai, K. and J. Lutkenhaus. 1992. The Proper Ratio of FtsZ to FtsA Is Required for Cell Division To Occur in Escherichia coli. J. Bacteriol. 174: 6145-6151.

Doerrler WT, Gibbons HS, Raetz CR. 2004. MsbA-dependent translocation of lipids across the inner membrane of Escherichia coli. J Biol Chem. 279; 45102-9.

Guatam, A., M. Hathaway, and R. Ramamoorthy. 2009. The *Borrelia burgdorferi flaB* promoter has an extended -10 element and includes a T-rich -35/-10 spacer sequence that is essential for optimal activity. FEMS Microbiol. Lett. 293: 278-284.

Hovius, J. W., A. P. van Dam, and E. Fikrig. 2007. Tick-host-pathogen interactions in Lyme borreliosis. Trends Parasitol 23:434-8.

Liang, F.T., Q. Xu, R. Sikdar, Y. Xiao, J. Cox, and W. Doerrler. 2010. BB0250 of Borrelia burgdorferi is a Conserved and Essential Inner Membrane Protein Required for Cell Division. J. Bacteriol. 192: 6105-6115.

Purser, J., M. Lawrenz, M. Caimano, J. Howell, J. Radolf, and S. Norris. 2003. A plasmid-encoded nicotinamidase (PncA) is essential for infectivity of *Borrelia burgdorferi* in a mammalian host. Mol. Microbiol. 48: 753-764.

Sikdar R and Doerrler WT. Inefficient Tat-Dependent Export of Periplasmic Amidases in an Escherichia coli Strain with Mutations in Two DedA Family Genes. J Bacteriology 192; 807-818, 2010.

Strom, M., and R. Paranjpye. 2000. Epidemiology and Pathogenesis of Vibrio vulnificus. Microbes and Infection. 2: 177-188.

Thompkins, K., B. Chattopadhyay, Y. Xiao, M.C. Henk, and W.T. Doerrler. 2008. Temperature sensitivity and cell division defects in an *Escherichia coli* strain with mutations in *yghB* and *yqjA*, encoding related and conserved inner membrane proteins. J. Bacteriol. 190: 4489-4500.

Wang, R.F., and S.F. Kushner. 1991. Construction of versatile low-copy-number vectors for cloning, sequencing and gene expression in *Escherichia coli*. Gene 100:195-199.

Xu, Q., K. McShan, and F.T. Liang. 2007. Identification of an *opsC* operator critical for immune evasion of *Borrelia burgdorferi*. Mol. Microbiol. 64: 220-231.

Table 1. Number of *dedA* family genes (amino acid BLAST e-value < 0.02) found in sequenced genomes of representative bacterial and archaeal species. Significant homologs (Protein BLAST E-value < 0.02) of *E. coli* YqjA, YghB, DedA, YohD, YabI, YdjZ, YdjX or YqaA were included in the number of genes displayed in the second column (Liang et al, 2010). Genomes displayed in red were analyzed in this study. Accession numbers for each gene are listed in Table 4.

Strain	**# DedA family homologs**
Escherichia coli K12	**8**
Vibrio vulnificus YJ016	**2**
Sinorhizobium meliloti 1021	**2**
Borrelia burgdorferi B31	**1**
Salmonella enterica SL480	**6**
Pseudomonas aeruginosa PAO1	**5**
Helicobacter pylori J99	**2**
Vibrio cholerae El Tor N16961	**3**
Caulobacter crescentus CB15	**3**
Neisseria meningitidis Z2491	**3**
Bacillus subtilus str. 168	**6**
Bacillus anthracis str. Ames	**8**
Mycobacterium tuberculosis H37Rv	**4**
Chlamydia trachomatis D/UW-3/CX	**0**
Synechocystis sp. PCC6803	**3**
Halobacterium salinarum NRC-1	**1**

Table 2. PCR primers used in this study.

Primer name	**Primer sequence**
250F	5'-GCGCCATATGACAAAAATGTATATAAACACAATAATAG-3' (NdeI site underlined)
250R	5'-CCCGGGATCCTTATTTTAAATTTTTGTCAACTTTTTTATTTTTTTTAATTAC-3' (BamHI)
250GF	5'-TAATACGACTCACTATAGGG-3' (T7 promoter primer)
250GR	5'-GCCTCGAGTTTTAAATTTTTGTCAACTTTTTTATTTTTTTTAATTACGTAG-3' (XhoI)
P1F	TAGGATCCGGATCCAAGATAGAGAGAGAAAGTG (BamHI)
P1R	GCTCTAGATTCCTCCATGATAAAATTTAAATTTCTGAC (XbaI)
P2F	GCTCTAGAGTGTCTAGGAATTTGATAGTAGGTTTAG (XbaI)
P2R	GGCTCGAGATCATCGCTACTTTTTTTATTTAAATTTC (XhoI)
P3F	5'-GCCATATGAGCCATGGTATCAGCAAC-3' (NdeI)
P3R	5'-TAGGATCCGCCTCCGTCATGCACCTTTG-3' (BamHI)
P4F	5'-GACATATGGAGTTTTTTACTGCGCTAATC-3' (NdeI)
P4R	5'-GACGGATCCTTATCGTTGGTTGAAATAACGAATG-3' (BamHI)
P5F	5'-GATCTAGAATGGAGTTTTTTACTGCGCTAATC (XbaI)
P5R	5'-GACTCGAGTTATCGTTGGTTGAAATAACGAATG (XhoI)

Table 3. Bacterial Strains and Plasmids

Strain or plasmid	Relevant genotype or description	Source of reference
Strains		
E. coli W3110	Wild type; $F^- \lambda^-$	*E. coli* genetic stock center, Yale University
E. coli BC202	W3110 *ΔyqjA::Tet*^{r} *ΔyghB781::Kan*^{r}	Thompkins et al., 2008
B. burgdorferi 13A	*B. burgdorferi* B315A13 subculture with high transformability and lacking plasmids lp25 and lp56	Xu et al., 2007
Plasmids		
pET28b	Expression vector, T7 *lac* promoter; Kan^r	Novagen
pET-BB0250	BB0250 cloned into NdeI/BamHI sited of pET28	This work
pTB28	Ami-C GFP expression vector; Amp^r	Bernhardt and de Boer, 2003
pBB0	GFP expression vector, based on pTB28; Amp^r	This work
pBB0250-GFP	BB0250-GFP expression vector; pTB28 with *bb0250* cloned into XbaI/XhoI sites (following removal of *amiC*); Amp^r	This work
pBBE22	Shuttle vector ;Kan^r	Purser et al., 2003
pWSK29	Expression vector; *lac* promoter, Amp^r	Wang et al., 1991
pJC2	pWSK29 with *smc01718* cloned into BamHI/XbaI sites	This work

Table 4. Accession numbers for genes referred to in Table 1. Accession numbers of *dedA* family genes (amino acid BLAST e-value < 0.02) found in sequenced genomes of representative bacterial and archaeal species. Significant homologs (Protein BLAST E-value < 0.02) of *E. coli* YqjA, YghB, DedA, YohD, YabI, YdjZ, YdjX or YqaA were included in the number of genes displayed in the second column (Liang et al., 2010).

Species	Accession #
Escherichia coli K12 MG1655	NP_416820.1 (DedA) NP_417566.1 (YqjA) NP_417482.1 (YghB) NP_414607.1 (YabI) NP_416640.2 (YohD) NP_416264.4 (YdjX) NP_416266.1 (YdjZ) NP_417174.1 (YqaA)
Salmonella typhimurium LT2	NP_461309.1 NP_462140.1

	NP_462077.1 NP_459110.1 NP_461115.1 NP_461745.1
Pseudomonas aeruginosa PAO1	NP_252718.1 NP_252700.1 NP_253931.1 NP_249900.1 NP_251442.1
Helicobacter pylori J99	NP_223236.1 NP_222938.1
Vibrio cholerae El Tor N16961	NP_233040.1 NP_231359.1 NP_230204.1
Caulobacter crescentus CB15	NP_420509.1 NP_420349.1 NP_419348.1
Neisseria meningitidis Z2491	YP_002342627.1 YP_002343221.1 YP_002342168.1
Borrelia burgdorferi B31	NP_212384.1
Bacillus subtilis str. 168	NP_388110.1 NP_389226.1 NP_389701.1 NP_390775.1 NP_390449.1 NP_388929.1
Bacillus anthracis str. Ames	NP_842958.1 NP_847451.1 NP_843328.1 NP_845187.1 NP_844450.1 NP_847272.1 NP_843623.1 NP_843133.1
Synechocystis sp. PCC6803	NP_442167.1 NP_442514.1 NP_442601.1
Halobacterium salinarum NRC-1	NP_280417.1

Figures

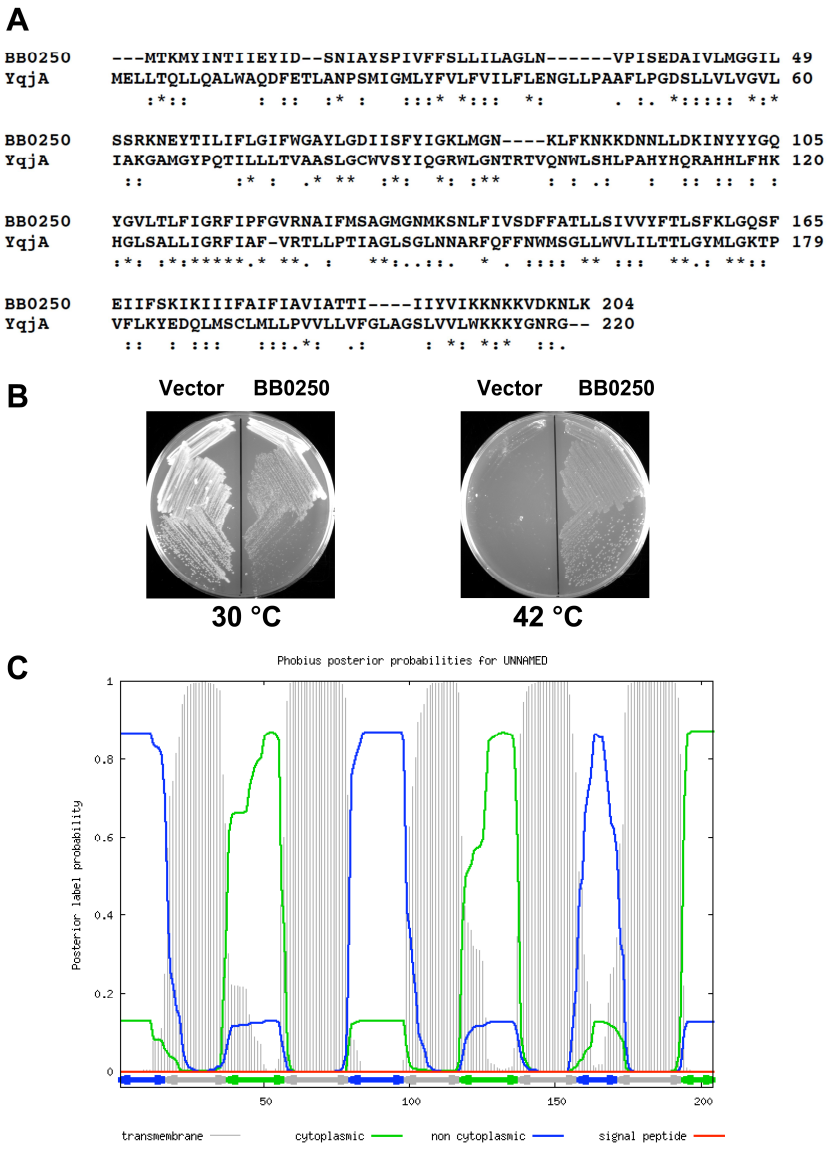


Figure 1. (A) ClustalW alignment of *Borrelia burgdorferi* BB0250 and *E. coli* YqjA. The two proteins display 19% identity and 56% similarity. (B) BC202 transformed with control vector and pBB0250-GFP streaked on plates containing ampicillin and 1mM IPTG. pBB0250-GFP restores growth to BC202 at 42°C (Liang et al. 2010). (C) Predicted membrane topology of BB0250 using Phobius software. Each grey peak represents a predicted membrane-spanning α-helix. BB0250 is predicted to contain 5 such membrane spanning domains with the C-terminus of the protein facing the cytoplasm.

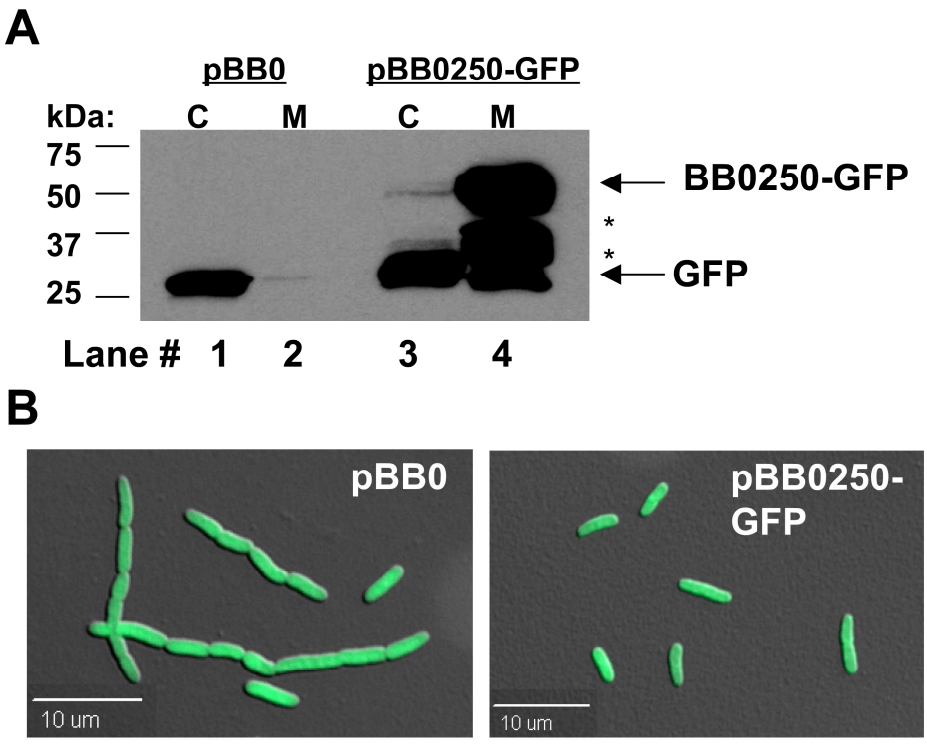


Figure 2. (A) BC202 transformed with control plasmid pBB0 (expressing GFP alone) or pBB0250-GFP grown in LB broth containing 300μM IPTG. Western Blot of cytoplasmic (C) and Membrane (M) fractions using anti-GFP antibody showed BB0250-GFP correctly localized to the membrane. (B) Microscopy of BC202 containing either pBB0 or pBB0250-GFP grown at 30°C in LB broth containing 300μM IPTG. The bright fluorescence seen in cells expressing BB0250-GFP is likely due to substantial levels of free GFP found in the cytoplasm (see panel A) of these cells likely due to proteolysis of the fusion protein in E. coli. However, BB0250 does correct the cell division defect of BC202.

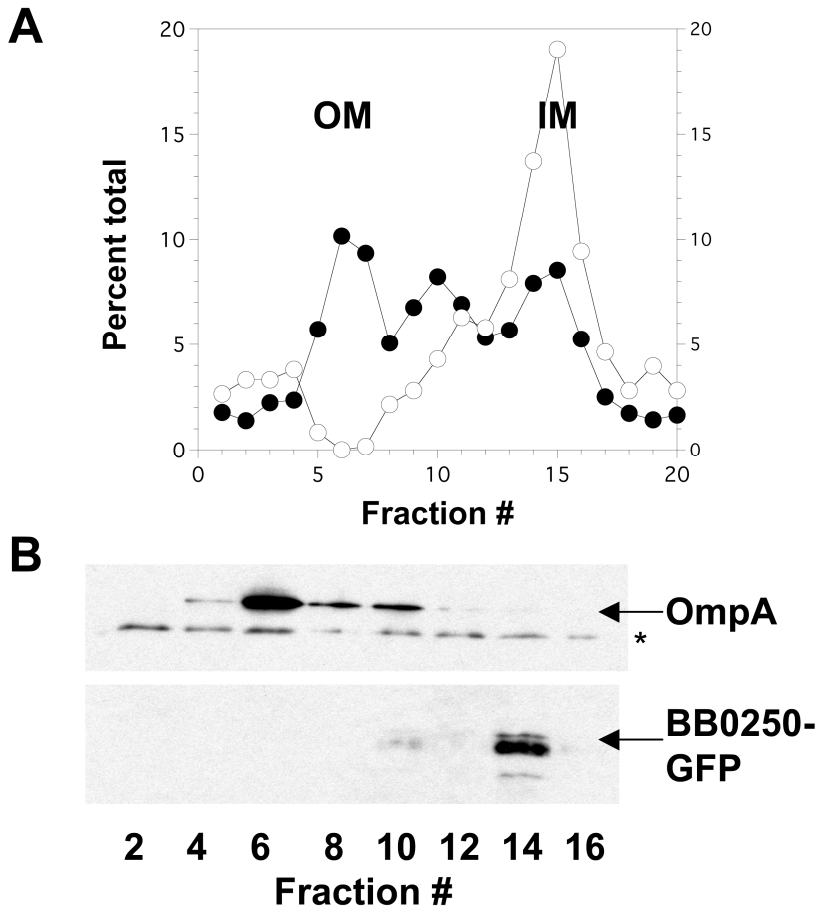


Figure 3. (A) Membranes of BC202 transformed with pBB0250-GFP were separated using a 12 ml, 30-60% isopycnic sucrose gradient and 0.5 ml fractions were collected. Filled circles represent total protein levels. Open circles represent NADH oxidase activity, a marker for the inner membrane. (B) Western Blot of fractions using antibodies against outer membrane protein OmpA (top panel) and antibodies against GFP showing BB0250-GFP in the inner membrane.

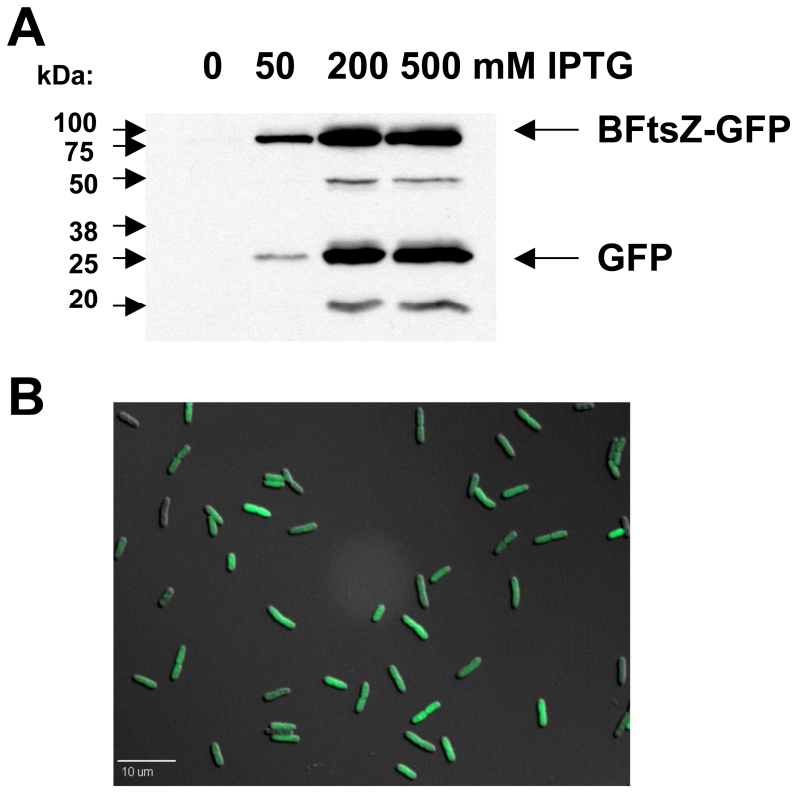


Figure 4. (A) *E. coli* W3110 transformed with pTB28 expressing *Borrelia* FtsZ-GFP grown in 0, 50, 200, and 500 μM IPTG and a Western blot performed using anti-GFP antibody. Arrows indicate bands representing Borrelia burgdorferi FtsZ-GFP (BFtsZ-GFP) and GFP, presumably produced by degradation of the larger fusion protein. (B) DIC and fluorescent microscopy overlay of *E. coli* W3110 expressing *Borrelia* FtsZ-GFP grown at 30 °C with ampicillin and 100 μM IPTG.

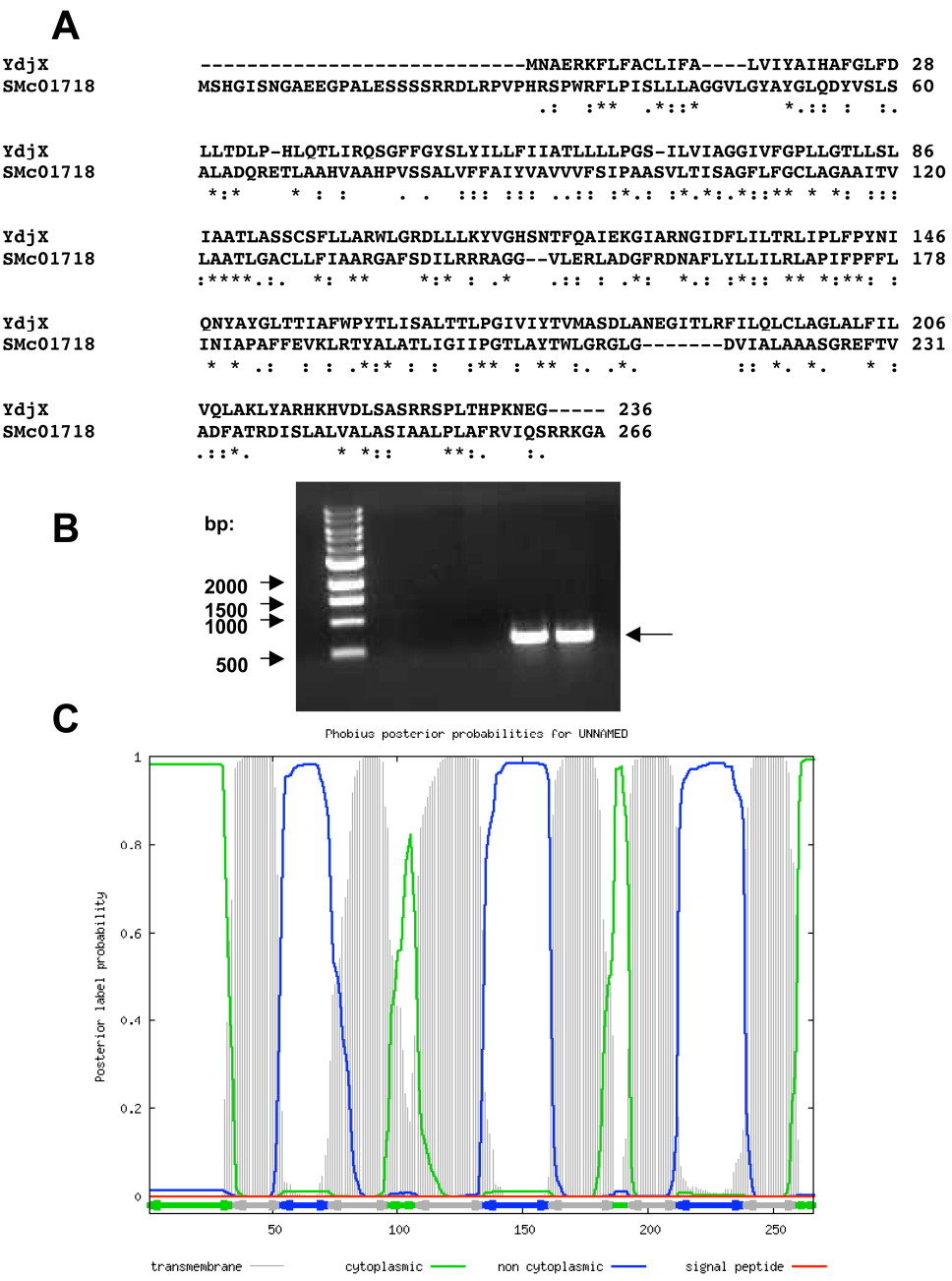
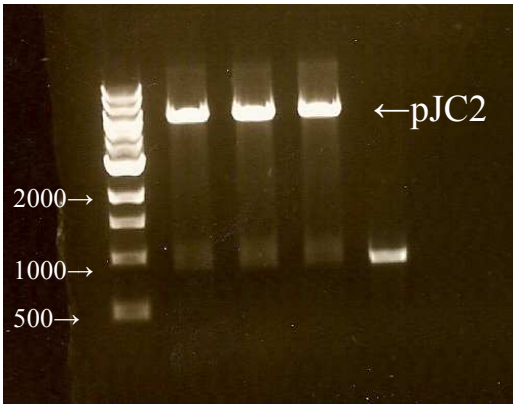


Figure 5. Analysis of *Sinorhizobium meliloti* 1021 DedA family member, annotated SMc01718. (A) ClustalW alignment between SMc01718 and *E. coli* YdjX. The two proteins display 22% amino acid identity across the entire sequences. (B) PCR amplification of *smc01718*. (C) Predicted membrane topology of Smc01718 using Phobius software.

A



B

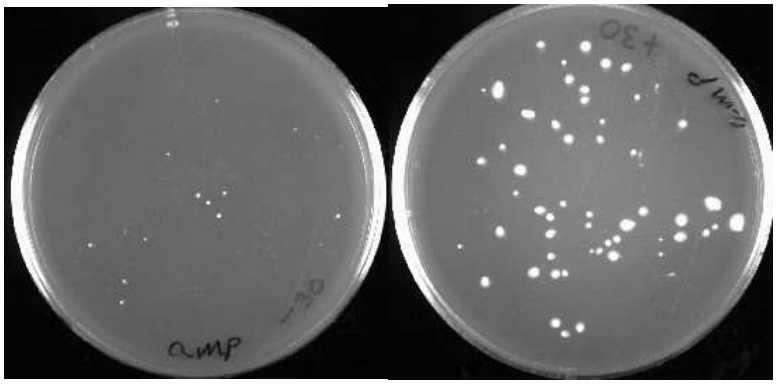


Figure 6. (A) Ligation of *smc01718* into pWSK29, forming pJC2. (B) BC202 transformed with vector and pJC2 spread onto plates containing ampicillin and 250μM IPTG. pJC2 forms larger colonies with mucoid appearance.

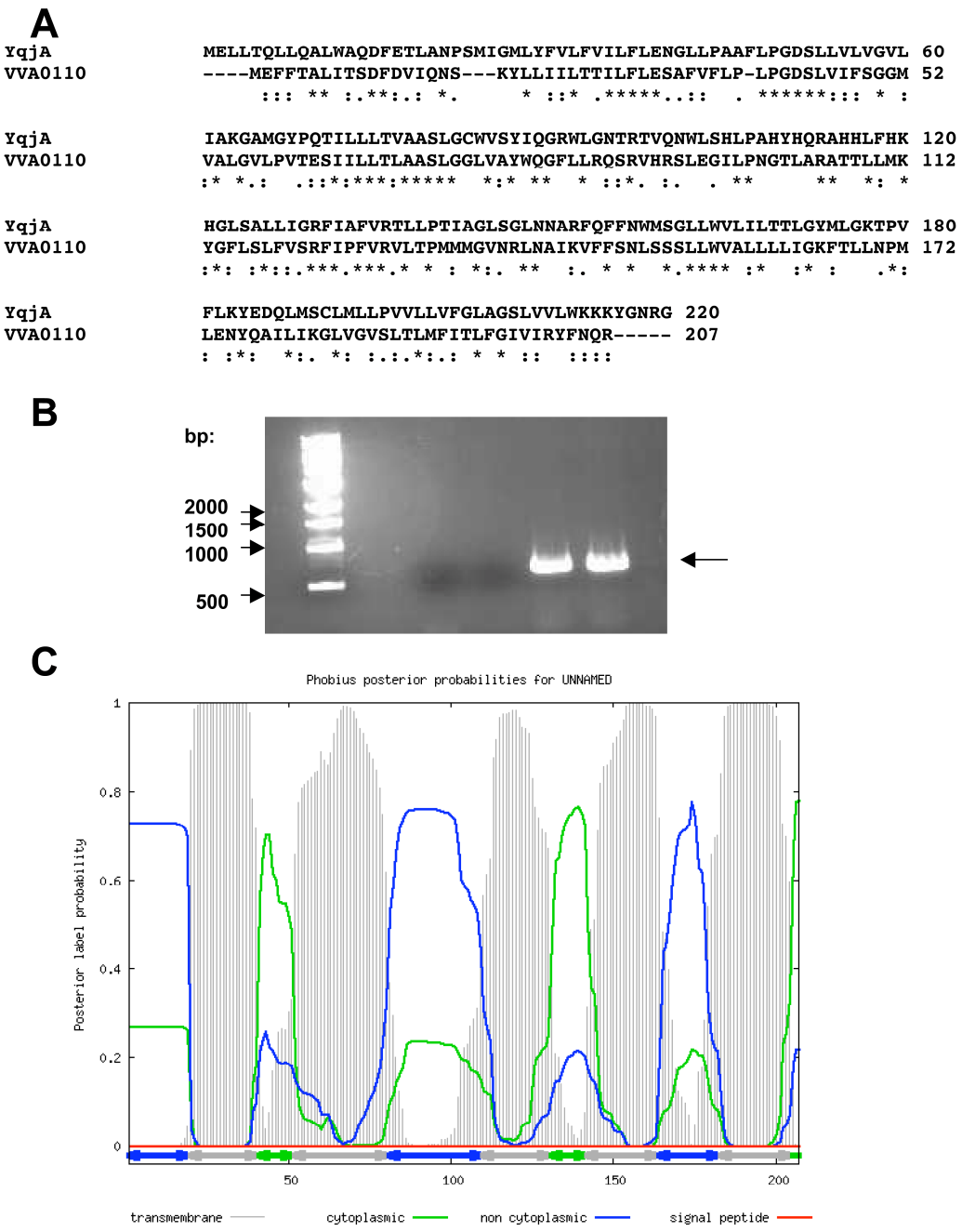


Figure 7. Analysis of *Vibrio vulnificus* YJ016 DedA family member, annotated VVA0110. (A) ClustalW alignment between VVA0110 and *E. coli* YqjA. The two proteins display 32% amino acid identity across their entire sequences. (B) PCR amplification of *vva0110*. (C) Predicted membrane topology of VVA0110 using Phobius software.