Identiﬁcation of essential arginine residues of Escherichia coli DedA/Tvp38 family membrane proteins YqjA and YghB

Sujeet Kumar  
*Louisiana State University*

Cersten L. Bradley  
*Louisiana State University*

Patience Mukashyaka  
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William T. Doerrler  
*Louisiana State University*

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**Recommended Citation**
Identification of essential arginine residues of *Escherichia coli* DedA/Tvp38 family membrane proteins YqjA and YghB

Sujeet Kumar, Cersten L. Bradley, Patience Mukashyaka and William T. Doerrler*

Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803, USA

*Corresponding author: Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803, USA. Tel: +225-578-7904; E-mail: wdoerr@lsu.edu

One sentence summary: Identification of essential arginines in a DedA/Tvp38 family protein consistent with a putative transporter function.

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**ABSTRACT**

*Escherichia coli* DedA/Tvp38 family proteins YghB and YqjA are putative membrane transporters with 62% amino acid identity and overlapping functions. An *E. coli* strain (BC202) with nonpolar ΔyghB and ΔyqjA mutations displays cell-division defects and temperature sensitivity and is sensitive to antibiotics and alkaline pH. In this study, we performed site-directed mutagenesis on conserved, charged amino acids of YqjA and YghB. We discovered two conserved predicted membrane-embedded arginines (R130 and R136) that are critical for function in both proteins as defined by their ability to complement BC202 phenotypes, when expressed from a plasmid. Lysine can substitute for arginine at position R130 indicating a charge dependence at this position, but could not substitute at R136. In light of the established role that arginine plays in the translocation mechanism of numerous membrane transporters, we hypothesize that these amino acids play a role in the transport mechanism of these DedA/Tvp38 family proteins.

**Keywords:** membrane transport; site-directed mutagenesis; antibiotic resistance; alkaline tolerance

**INTRODUCTION**

The DedA/Tvp38 family is a highly conserved and poorly understood family of membrane proteins present in most bacteria as well as many archaea and eukaryotes (Doerrler et al. 2013). Computational analysis has suggested DedA family proteins share an evolutionary relationship and structural fold with LeuT, belonging to the neurotransmitter: sodium symporter family (Khafizov et al. 2010; Keller, Ziegler and Schneider 2014). Our laboratory has pioneered characterization of the DedA/Tvp38 membrane protein family in *Escherichia coli*. (Thompkins et al. 2008; Liang et al. 2010; Sikdar and Doerrler 2010; Boughner and Doerrler 2012; Sikdar, Simmons and Doerrler 2013; Kumar and Doerrler 2014, 2015).

YqjA and YghB of *E. coli* are the best characterized members of the DedA family. YqjA and YghB possess 220 and 219 amino acids, respectively, along with multiple predicted membrane-spanning domains, sharing 62% amino acid identity and partially-redundant functions (Thompkins et al. 2008). Simultaneous in-frame deletions of these two genes in strain BC202 result in numerous phenotypes including temperature sensitivity (Thompkins et al. 2008), cell-division defects due to inefficient export of periplasmic amidases by the twin arginine pathway (Sikdar and Doerrler 2010) and sensitivity to various antibiotics and biocides which are normally effluxed in a protonmotive force (PMF)-dependent manner from the cytoplasm (Kumar and Doerrler 2014). Phenotypes of BC202 can be rescued in planktonic cultures by expression of wild-type YqjA and YghB from a plasmid (Kumar et al. 2015). Simultaneous deletion of *yqjA* and *yghB* (BC202) results in numerous phenotypes, including temperature sensitivity (Thompkins et al. 2008), cell-division defects due to inefficient export of periplasmic amidases by the twin arginine pathway (Sikdar and Doerrler 2010), sensitivity to various antibiotics and biocides which are normally effluxed in a protonmotive force (PMF)-dependent manner from the cytoplasm (Kumar and Doerrler 2014). Phenotypes of BC202 can be rescued in planktonic cultures by expression of wild-type YqjA and YghB from a plasmid (Kumar et al. 2015).
by growth at pH 6.0 or overexpression of either yqjA, yghB or mdfA (Sikdar, Simmons and Doerrler 2013; Kumar and Doerrler 2014). MdfA is an unrelated Na+–K+/H+ antiporter and drug efflux pump belonging to the major facilitator superfamily (Lewinson et al. 2003).

The ΔyghA mutant (but not the ΔyghB mutant) is unable to survive above pH 8.5 and sodium or potassium is required for YqjA-mediated alkaline tolerance (Kumar and Doerrler 2015). Cation/proton antiporters play a major role in alkaline pH homeostasis in E. coli (Pinner et al. 1993; Lewinson, Padan and Bibi 2004; Padan et al. 2005; Holdsworth and Law 2013). Based on these results, we suggested YqjA/YghB are proton-dependent membrane transporters (Sikdar, Simmons and Doerrler 2013; Kumar and Doerrler 2014, 2015).

Charged amino acids are known to play essential roles in various secondary transporters including MdfA, NhaA, MdtM and LacY (Gerchman et al. 1993; Noumi et al. 1997; Abramson, Iwata and Kaback 2004; Adler and Bibi 2004; Sigal et al. 2005; Fluman et al. 2012; Holdsworth and Law 2012). Membrane-embedded acidic amino acids were shown to be important for the function of YqjA and YghB of E. coli (E39 and D51 of both proteins) (Kumar and Doerrler 2014). Furthermore, the significance of membrane-embedded basic amino acids like arginine is well documented in the literature and shown to be crucial in several biological processes including regulation of redox potential (Cutler et al. 1989; Winn et al. 2002), voltage detection across a lipid bilayer (Jiang et al. 2003; Long, Campbell and Mackinnon 2005; Tao et al. 2010) and proton transport (Cain and Simoni 1989; Helmer, Teubner and Zeilinger 2003; Sigal et al. 2005). We hypothesized that YqjA and YghB would possess critical basic amino acids and these would be found at similar positions in both proteins. Here, we analyze the effect of point mutations of conserved basic amino acids on the function of YqjA and YghB and show that conserved arginines at positions 130 and 136 of YqjA and YghB are essential for the function of both proteins. Furthermore, mutation of R130 to lysine results in a functional protein indicating a charge dependence at this position. In contrast, the R136K mutation remains nonfunctional indicating an important role played by arginine at this position. This study provides mechanistic insight into YqjA and YghB and supports the hypothesis that they function as membrane transporters.

MATERIALS AND METHODS

Materials

All chemicals were reagent grade and purchased from Sigma-Aldrich (St. Louis MO), VWR (Radnor PA), New England Biolabs (Ipswich MA) or Qiagen (Hilden, Germany).

Bacterial growth conditions

Bacteria cultures were grown in Luria-Bertani (LB) medium (1% tryptone, 0.5% yeast extract and 1% NaCl) with antibiotics ampicillin (Amp) at 100 µg/ml, kanamycin (Kan) at 30 µg/ml and tetracycline (Tet) at 12.5 µg/ml where specified. In certain experiments, growth media were additionally supplemented with 0.002% or 0.02% arabinose (w/v). For pH experiments, the growth media were buffered with 70mM BTP (Bis-Tris propane) and pH was adjusted as required with HCl.

Site-directed mutagenesis

Point mutants were created according to a previously published protocol (Kumar and Doerrler 2014). The point mutation was introduced using primers carrying the site-specific mutations (Table S2, Supporting Information) in a polymerase chain reaction (PCR) to amplify a vector having the indicated wild-type gene. The DpnI-digested PCR products were used to transform competent XL1 blue cells. Amp-resistant colonies were screened by colony PCR by means of gene-specific primers. Mutations were confirmed by deoxyribonucleic acid (DNA) sequencing conducted at the LSU College of Science Genomics Facility.

Microscopy

Microscopy was performed as previously described (Kumar and Doerrler 2014). Overnight cultures of specified strains were diluted 1:100 in LB media with appropriate antibiotics and additives, and grown shaking to OD_{600} ~0.6 at 30 °C. The cells were resuspended to a final OD_{600} of 1.0 and 10 µl of cells were used for imaging on a 1% agarose coated glass slide. Differential interference contrast micrographs were obtained using a Leica DM-RXA2 deconvolution microscope (LSU Shared Instrument Facility).

Drug resistance measurements

Growth was carried out on solid media as described (Kumar and Doerrler 2014). Overnight cultures of E. coli were diluted 1:100 into LB media containing Amp and 0.1% arabinose and grown to OD_{600} ~0.6 at 30 °C. A total of 5 µl of serially log_{10}-diluted cells were spotted on LB agar plates containing 0.002% arabinose and the indicated drugs/biocides. Plates were incubated at 30 °C for 20–24 h.

Alkaline pH sensitivity

Alkaline pH sensitivity was tested as previously described (Kumar and Doerrler 2015). Overnight cultures of E. coli were diluted 1:100 in LB media with appropriate antibiotics and additives, and grown to OD_{600} ~0.6 at 37 °C. A total of 5 µl of serially log_{10}-diluted cells were spotted on LB agar plates at the indicated pH with appropriate antibiotics and additives. Plates were incubated at 37 °C for 20–24 h. All experiments were repeated three times.

Membrane preparation and western blotting

Isolation of membranes and western blotting were performed as previously described (Kumar and Doerrler 2014). Cell membranes were prepared from the log phase cultures of BC202 expressing the indicated proteins. Protein samples were incubated with 2X sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS-PAGE) buffer and heated at 95 °C for 5 min before 12% SDS-PAGE and transfer to polyvinylidene difluoride (PVDF). Western blotting was done using Penta-His primary antibody (Qiagen, Hilden, Germany) at 1:5000 dilution and goat-anti-mouse IgG horseradish peroxidase secondary antibody (Pierce, Thermo-Fisher Scientific; Waltham MA) at 1:1 000 and detection with the ImmunStar HRP kit (Bio-Rad; Hercules, CA).

RESULTS

Identification of amino acids required for YqjA and YghB function

Numerous studies have confirmed the role of charged amino acids in the transport mechanism of membrane transporters (Gerchman et al. 1993; Wiebe, Dibattista and Fliegel et al. 2001; Adler and Bibi 2004; Sigal et al. 2005; Holdsworth and Law 2012). Therefore, we investigated the significance of conserved,
Figure 1. Amino acid alignment and hydrophobicity analysis of YqjA and YghB and summary of mutation data. (A) Amino acid alignment of YqjA and YghB. ClustalW (Larkin et al. 2007) was used to create an alignment between the two protein sequences. The charged amino acids which are conserved between both the proteins were chosen for site-directed mutagenesis study. Essential acidic (blue) and basic (red) residues are highlighted. (B) Predicted membrane topology model of YqjA/YghB based on the TMHMM program (Sonnhammer, von Heijne and Krogh 1998). The amino acids E39 and D51 (white type, black background) were previously shown important for the function of YqjA/YghB (Kumar and Doerrler 2014). YqjA/YghB residues mutated in this study are represented as ovals. Mutation of YqjA/YghB R130, R136 or E185 results in loss of function (yellow type, black background). Mutation of YqjA D15, H121 and YqjA/YghB D186 results in proteins with wild-type activity (black type, white background). Asterisk indicates mutation of YqjA/YghB E185 results in a partial loss of function in certain assays (See Figs 2 and 3). All other amino acids shown in the model are non-essential for the function of YqjA (Keller et al. 2015). (C) A hydrophobicity profile of YqjA and YghB. The profiles were generated by using the AlignMe program (Khafizov et al. 2010; Stamm et al. 2013; Stamm et al. 2014) along with the similarity scale from Hess, White and van Heinje (HvWvH) with a threshold for gap penalty of −0.5. The green and red plots represent the hydrophobicity profile of YghB and YqjA, respectively.

<table>
<thead>
<tr>
<th>Charged Residues in YqjA and YghB</th>
<th>Summary of Mutation Data</th>
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<tbody>
<tr>
<td>YghB MHLQDD14FLFAASGLGDSLILAGS</td>
<td>Loss of function (R130, R136, E185)</td>
</tr>
<tr>
<td>YqjA MHLQDD14FLFAASGLGDSLILAGS</td>
<td>Wild-type activity (D15, H121)</td>
</tr>
</tbody>
</table>

**Figure 1.** Amino acid alignment and hydrophobicity analysis of YqjA and YghB and summary of mutation data. (A) Amino acid alignment of YqjA and YghB. ClustalW (Larkin et al. 2007) was used to create an alignment between the two protein sequences. The charged amino acids which are conserved between both the proteins were chosen for site-directed mutagenesis study. Essential acidic (blue) and basic (red) residues are highlighted. (B) Predicted membrane topology model of YqjA/YghB based on the TMHMM program (Sonnhammer, von Heijne and Krogh 1998). The amino acids E39 and D51 (white type, black background) were previously shown important for the function of YqjA/YghB (Kumar and Doerrler 2014). YqjA/YghB residues mutated in this study are represented as ovals. Mutation of YqjA/YghB R130, R136 or E185 results in loss of function (yellow type, black background). Mutation of YqjA D15, H121 and YqjA/YghB D186 results in proteins with wild-type activity (black type, white background). Asterisk indicates mutation of YqjA/YghB E185 results in a partial loss of function in certain assays (See Figs 2 and 3). All other amino acids shown in the model are non-essential for the function of YqjA (Keller et al. 2015). (C) A hydrophobicity profile of YqjA and YghB. The profiles were generated by using the AlignMe program (Khafizov et al. 2010; Stamm et al. 2013; Stamm et al. 2014) along with the similarity scale from Hess, White and van Heinje (HvWvH) with a threshold for gap penalty of −0.5. The green and red plots represent the hydrophobicity profile of YghB and YqjA, respectively.
Mutations were introduced into YqjA at six different positions (D15, H121, R130, R136, E185 or D186) and YghB at four positions (R130, R136, E185 and D186). All genes were cloned into vector pBAD in-frame with an N-terminal His tag, which allows detection of expression and membrane association of proteins using western blot. The vector with the desired mutation in yqjA or yghB was transformed into BC202 or ΔyqjA to study its ability to complement certain mutant phenotypes.

Rescue of temperature sensitivity of BC202 by point mutants

We first tested the ability of the mutant proteins to complement temperature sensitivity of BC202, which stops growing and releases cytoplasmic contents following a short growth at 44°C (Thompkins et al. 2008). Of the six YqjA mutants, YqjA-D15A, -H121A and -D186A were functional, able to support growth of BC202 at 42°C (Fig. 2A, top panel). Of the four YghB mutants, only YghB-D186A supported growth at 42°C (Fig 2C, top panel). All mutants are well expressed in membranes indicating proper folding of the expressed mutant proteins with the exception of YqjA-D15A (Fig 2B), in spite of its ability to complement BC202 at 42°C (Fig. 2A). While the reasons for poor expression are unclear, residue D15 appears to be dispensable for YqjA function from our data.

Since the R130A and R136A mutations of YqjA and YghB failed to rescue growth of BC202, we constructed R130K and R136K mutants replacing the positively charged arginine with positively charged lysine to distinguish between the effect of size or charge in their loss of function. Notably, the R130K but not the R136K mutant of both YqjA and YghB regain their ability to support growth of BC202 at 42°C (Fig. 2A and C). This suggests that amino acid charge at position 130 is critical for the function of both these proteins. However, arginine is absolutely required at position R136, as lysine cannot substitute. YqjA-E185A and YghB-E185A also fail to support growth of BC202 at 42°C (Fig. 2A and C). These mutants gave contradictory results in some of our assays and are discussed in more detail below.

Rescue of drug sensitivity of BC202 by point mutants

BC202 is also sensitive to several biocides and antibiotics which are normally effluxed from the cell via PMF-dependent pumps including EmrE, MdfA and AcrB (Kumar and Doerrler 2014). BC202 transformed with control vector forms long chains of incompletely constricted cells (Fig. 3A and B) as previously reported (Thompkins et al. 2008). BC202 expressing a plasmid copy of yqjA or yghB regains normal cell division and there is no sign of elongated cells. YqjA-D15A, -H121A and -D186A were functional in a way that they were each capable of supporting cell division of BC202 while YqjA-R130A, -R136A and E185A were nonfunctional. When we made arginine-to-lysine substitutions at positions 130 and 136 of YqjA, we again found that YqjA-R130K regained function but not YqjA-R136K, suggesting an absolute dependence upon arginine at position 136 of YqjA (Fig. 3A). Similar observations were made in the corresponding YghB mutants with the exception of YghB-E185A, which was functional in a way that it did support normal cell division of BC202 (Fig. 3B).

Rescue of alkaline sensitivity of ΔyqjA by point mutants

The E. coli ΔyqjA mutant (but not the ΔyghB mutant) cannot grow at a pH exceeding 8.5 (Kumar and Doerrler 2015). Plasmid copies of yqjA or yghB rescue growth of ΔyqjA up to pH 9.5 or 9.0, respectively. We therefore tested each point mutant for their
ability to rescue growth of the ΔyqjA mutant at elevated pH (Fig. 5). Our results largely agree with the results for BC202 in Figs 2–4, in that only the R130A and R136A mutants of YqjA and YghB are nonfunctional in that they fail to support growth of ΔyqjA under alkaline conditions. Again, lysine can substitute for arginine at position 130 but not 136 in each protein (Fig. 5A and B).

To our surprise, we found a discrepancy in the ability of YghB-E185A to rescue BC202 compared to the YqjA-E185A mutant. While YqjA-E185A rescues drug (Fig. 4A) and pH sensitivity (Fig. 5A), it fails to rescue temperature sensitivity (Fig. 2A) and cell division (Fig. 3A). In contrast, YghB-E185A corrects the drug sensitivity (Fig. 4B) and cell-division defect (Fig. 3B) of BC202 and the pH sensitivity of ΔyqjA (Fig. 5B), while not correcting the temperature sensitivity of BC202 (Fig. 2C). All mutant proteins are expressed well in the membrane fraction, indicating proper folding and targeting (Fig. 2B and D). The reasons for the differing ability of E185A mutants of YqjA and YghB to rescue cell division are at present unclear, but both mutants are classified here as partially functional.

**DISCUSSION**

YqjA and YghB belong to the highly conserved DedA/Tvp38 membrane protein family of *E. coli* (Doerrler et al. 2013). Previously, we characterized the importance of YqjA/YghB in growth at elevated temperatures, cell division, drug resistance and alkaline pH tolerance in *E. coli* and have suggested that they possess properties suggesting they function as membrane transporters (Thompkins et al. 2008; Sikdar and Doerrler 2010; Doerrler et al. 2013; Kumar and Doerrler 2014, 2015). YqjA and YghB share 62% amino acid identity and partially-redundant functions (Thompkins et al. 2008). In-frame deletions of both genes in the same strain (BC202) result in cell-division defects due to inefficient export of periplasmic amidases by the twin arginine transport pathway (Sikdar and Doerrler 2010) and drug sensitivity due to inefficient function of drug efflux pumps belonging to the RND (resistance-nodulation-division), MFS (major facilitator superfamily) and SMR (small multidrug resistance) protein families (Kumar and Doerrler 2014). The common denominator of these two phenotypes is their reliance upon the PMF and we have shown using a JC-1 dye based assay that the PMF is, in fact, poorly maintained in BC202 (Sikdar, Simmons and Doerrler 2013). In addition, YqjA is required for alkaline tolerance of *E. coli* as the ΔyqjA strain grows poorly at pH > 8.5 (Kumar and Doerrler 2015). YqjA requires divalent cations sodium or potassium to support growth of *E. coli* under alkaline conditions. While the ΔyghB strain is not alkaline pH sensitive, likely due to the presence of a functional YqjA, a plasmid copy of *yghB* can partially restore alkaline tolerance to the ΔyqjA strain (Kumar and Doerrler 2015). Finally, both YqjA and YghB possess acidic amino acids within their first transmembrane domain that are essential for function, similar in location to those found in proton-dependent transporters belonging to other families (Kumar and Doerrler 2014). These observations suggest YqjA, YghB and likely other members of the DedA/Tvp38 family may function as membrane transporters (Sikdar, Simmons and Doerrler 2013; Kumar and Doerrler 2014, 2015). In spite of our genetic and physiological evidence, mechanistic and structural details of this protein family remain unclear. A structural model of one DedA family member, Sir0305 of *Synechocystis* sp. PCC6803, has been published based upon a predicted similar fold to LeuT (see below) (Keller, Ziegler and Schneider 2014).

To provide a mechanistic insight into DedA family proteins, we introduced mutations of conserved and charged amino acids of YqjA and YghB and measured the effect of the mutant proteins on numerous phenotypes of BC202 and ΔyqjA. We observed that R130A and R136A mutations abolish the ability of YqjA and YghB to rescue BC202 and ΔyqjA suggesting an essential role of these amino acid in their function. In order to

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**Figure 3.** Correction of cell-division defects of BC202 by YqjA, YghB and point mutants. BC202 transformed with vector, pBAD-yqjA and YqjA point mutants (A) or vector, pBAD-yghB and YghB point mutants (B) were grown at 30°C in liquid media having 0.1% arabinose were visualized with a Leica DM-RXA2 deconvolution microscope. Bar = 10 μm.
distinguish whether the effect was due to changes in size or charge, we created the R130K and R136K variants of YqjA and YghB. We found that YqjA-130K and YghB-R130K regain full function suggesting that charge at this position is important for the function of both proteins. However, in spite of being well expressed, the R136K mutant of both the proteins was not able to complement the BC202 and ΔyqjA phenotypes implying that arginine at this position is essential for their function.

The importance of membrane-embedded arginines has been observed in a number of different families of membrane transporters including GlpT (Law et al. 2008), UhpT (Fann et al. 1998; Law et al. 2008), FOF1 ATPase (Cain and Simoni 1989), MjNhaP1 (Hellmer, Teubner and Zeilinger 2003), TetA (Kimura et al. 1998), MdtM (Holdsworth and Law 2012) and MdfA (Fann et al. 1998; Sigal et al. 2005; Law et al. 2008; Holdsworth and Law 2012). In these examples, arginine is thought to be directly involved in substrate binding, and faces the translocation channel.

MdfA appears to share some functional similarity to YqjA. Both the ΔyqjA and the ΔmdfA E. coli strains display alkaline sensitivity (Lewinson, Padan and Bibi 2004; Kumar and Doerrler 2014). Overexpression of MdfA is sufficient to suppress each mutant phenotype of BC202 and to partially confer pH resistance to ΔyqjA (Sikdar, Simmons and Doerrler 2013; Kumar and Doerrler 2014, 2015). Therefore, the importance of charged residues in the membrane domains of both proteins is potentially significant.

Acidic residues residing in the first transmembrane domain of both proteins (MdfA E26/D34 and YqjA/YghB E39/D51) have been shown to be functionally important (Fluman et al. 2012; Kumar and Doerrler 2014). In addition to these important negatively charged residues substitutions resulting in a loss of the positive charge at amino acid R112 in MdfA resulted in sensitivity to antibiotics (Sigal et al. 2005). It was suggested that R112 of MdfA could function in proton recognition (Sigal et al. 2005) similar to R302 of LacY (Abramson, Iwata and Kaback 2004). Recently, the inward facing substrate bound structure of E. coli MdfA was published and confirmed the importance of membrane-embedded R112 for transport activity (Heng et al. 2015).

LeuT, a member of the neurotransmitter: sodium symporter family appears to share structural features with DedA based upon computational analysis (Khafizov et al. 2010; Keller, Ziegler and Schneider 2014). It was hypothesized based upon this similarity that DedA proteins may form functional dimers in the membrane (Khafizov et al. 2010), and this hypothesis was recently confirmed using a crosslinking approach (Keller et al. 2015). A water mediated salt bridge formed between LeuT amino acids R30 and D404 is thought to form the extracellular gate (Noskov 2008; Singh et al. 2008; Krishnamurthy, Piscielli and Gouaux 2009). We propose, based on these studies that R130 and R136 of both YghB and YqjA are either exposed to the substrate translocation pore or play a role in substrate binding. An alternative possibility is that these residues form salt bridges with

Figure 4. Correction of drug sensitivity of BC202 by YqjA, YghB and point mutants. BC202 transformed with vector, pBAD-yqjA, and YqjA point mutants (A) and vector, pBAD-yghB, and YghB point mutants (B) was tested for sensitivity to acriflavine (25 μg/ml), benzalkonium chloride (40 μg/ml), ethidium bromide (100 μg/ml) and methyl viologen (30 μg/ml). All strains grew well on LB plates without biocides at 30°C.
negatively charged amino acids helping to maintain protein conformation during the transport cycle (Law et al. 2008). Their involvement in the specific transport mechanism may differ from those examples cited above and clarification awaits future structural studies. In conclusion, we have demonstrated that R130 and R136 are critical for the function of YqjA and YghB. These results will aid in elucidating the mechanism of transport of DedA/Tvp38 family members.

**SUPPLEMENTARY DATA**

Supplementary data are available at FEMSLE online.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


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**Conflict of interest**. None declared.

Figure 5. Correction of alkaline sensitivity of ΔyqjA by YqjA, YghB and point mutants. The ΔyqjA mutant transformed with vector, yqjA and YqjA point mutants (A) and vector, pBAD-yghB, and YghB point mutants (B) was grown to mid-log phase at pH 7.0 and 5 μl of serially diluted cells were spotted onto LB plates at pH 7.0, 9.0 and 9.25 (A) or pH 7.0, 8.75 and 9.0 (B). The plates were incubated at 37°C for 20–24 h. Growth at pH 7.0 was carried out with 0.002% arabinose and 0.02% arabinose was used at pH 9 and 9.25.


