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Complementation of *ompF* Into *ΔompCΔompF Escherichia coli* Confers Increased Sensitivity To SDS-EDTA Treatment

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SUMMARY The outer membrane (OM) of gram negative bacteria contributes to inherent antibiotic resistance as well as protection against toxins. OM general diffusion porins, such as OmpC and OmpF, regulate movement of biomolecules in and out of the periplasm, preventing large and potentially harmful molecules from entering the bacteria. OmpC has also been shown to contribute to membrane asymmetry through the Mla pathway. Previous studies have found *E. coli* deficient of OmpC are sensitive to treatment with detergent sodium dodecyl sulfate (SDS) and ethylenediaminetetraacetic acid (EDTA) when compared to wild type. In contrast, double deletions of OmpC and OmpF (*ΔompCΔompF)* show comparable resistance to wild-type (WT) *E. coli*. Further studies into single *ompF* deletions have shown no appreciable difference in sensitivity to SDS-EDTA from wild-type. Here, we hypothesize expression of *ompF* in the absence of *ompC* will result in sensitivity towards SDS-EDTA. To test this, we transformed a vector containing *ompF* into *ΔompCΔompF* mutants, then compared their relative sensitivity to WT and *ΔompC* mutants over increasing EDTA concentrations. Our study found complementation of *ompF* back into *ΔompCΔompF* mutants induced an increased SDS-EDTA sensitivity. Our results implicate a dynamic relationship between OmpF and OmpC, where presence of OmpC and expression of *ompF* may influence OM stability.

INTRODUCTION

Escherichia coli general diffusion porins OmpF and OmpC are β-barrels embedded in the outer membrane (OM) of gram-negative bacteria. Their role in stopping large molecules from entering the periplasm affords the bacteria intrinsic antibiotic resistance (1). Meanwhile, OmpC allows *E. coli* to maintain OM asymmetry through interactions with the Mla pathway, an ATP-binding cassette transport system (3). OM asymmetry occurs when lipopolysaccharides (LPS) accumulate on the outer leaflet and phospholipids on the inner leaflet, and is known to provide membrane stability when the bacterium encounters toxic environments (1). While the role of OmpC in the Mla pathway is unknown, deletion of OmpC have resulted in accumulation of phospholipids indicating that OmpC may be required for the transport of phospholipids to the inner leaflet (4). Studies have also shown knockout of any Mla proteins generate *E. coli* mutants that are more sensitive to ethylenediaminetetraacetic acid (EDTA) (4). It is thought that chelation of divalent cations by EDTA exposes the negatively-charged LPS to each other. This results in charge repulsion causing shearing of the outer leaflet of the OM, which leads to OM degradation (5). Brief treatment of *E. coli* with low concentrations of EDTA has been shown to increase membrane permeability and is associated with little or no injury to viability, growth rate, or normal RNA and protein synthesis (5). Many studies have scrutinized the functions of OmpC and OmpF, including their potential roles in OM integrity and resistance towards membrane targeting compounds such as sodium dodecyl sulfate (SDS) and/or EDTA (7, 8).

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Previous works have shown OmpC knockouts (*⊿ompC*) have increased sensitivity towards EDTA, where percent growth as measured by optical density was significantly ($p<0.05$) lower than WT cells (4, 7-9). OmpF has also been observed to confer sensitivity towards antibiotics treatment (10, 11). Counterintuitively, Hartstein *et al.* (2016) found *⊿ompC⊿ompF* mutants are resistant to SDS-EDTA treatment, comparable to wild-type (WT) *E. coli*. Investigating this further, Atif *et al.* (2017) proposed OmpF itself may play a role in altering the sensitivity of *E. coli* to EDTA, but found *⊿ompF* mutants showed no difference in SDS-EDTA sensitivity compared to WT *E. coli*. From these previous findings, we propose a new dynamic between OmpC and OmpF. OmpF is sensitive to various membrane weakening compounds, but OmpC plays a protective role by contributing to membrane stability, thereby alleviating the sensitivity caused by OmpF. Hence under conditions where OmpC is not expressed, OmpF expression may induce *E. coli* sensitivity towards membrane targeting compounds.

Therefore, we hypothesized that OmpF expression in the absence of OmpC would result in increased sensitivity of *E. coli* to SDS-EDTA, and complementation of *ompF* back into an *⊿ompC⊿ompF* mutant would restore sensitivity to SDS-EDTA treatment. We cloned *ompF* into *⊿ompC⊿ompF* mutants using TOPO-TA cloning, where successful transformants were validated through DNA sequencing. Once complementation was confirmed, we compared its relative sensitivity towards EDTA against the WT, *⊿omp*C, and *⊿omp*C*⊿omp*F mutants. We also investigated whether our newly generated plasmid had a negative impact on growth rate of our new strain.

METHODS AND MATERIALS

Bacterial Strains. A list of *E. coli* strains used is shown Table 1. Strains BW25113 and KJ740 were grown in Lysogeny Broth (LB), while strain JW2203 was grown in LB with kanamycin at 50 ug/mL. CyFi740 was selected by growth in LB with ampicillin at 100 ug/mL. All strains were grown at 37ºC.

Colony PCR amplification of *rpoS, ompC, and ompF*. The Polymerase Chain Reaction utilized primers specific against *rpoS*, *ompC*, and *ompF* (primer sequences shown in Table 2). The *rpoS* primers were used as our positive control, while the *ompC* and *ompF* primers were used to validate strain genotype. Per each PCR tube, 1X buffer, 200 uM dNTP mixture, 10 uM of each primer (forward and reverse), 1.5 mM $MgCl_2$, and 2 units/reaction Platinum Taq polymerase (ThermoFisher Scientific, Cat#10966018) were added. H_2O was added for a final volume of 50 µl. Using a sterile toothpick, one colony of each strain was picked and added to the appropriate PCR tube. For the *ompF* PCR, the reaction was held at 95ºC for 3 minutes, followed by 28 cycles of 95ºC for 0.5 minutes, 52ºC for 0.5 minutes, and 72ºC for 1 minute, then a final extension at 74ºC for 5 minutes. The *ompC* and *rpoS* reactions utilized the same parameters, except when primer annealing occurred at 50ºC for 0.5 minutes instead.

Agarose gel electrophoresis. A 1% agarose gel was made using 1X TAE buffer with 10,000X SYBR™ Safe DNA gel stain (ThermoFisher Scientific, Cat#S33102). DNA samples were mixed with 10X Loading Buffer then loaded onto the gel. The 1kb plus DNA ladder by

TABLE 1 Description of *Escherichia coli* strains used

Strain	Mutation	Reference/Source
BW25113	WT (none)	(12)
JW2203	*⊿ompC*	(13)
KJ740	*⊿ompC⊿ompF*	(14)
CyFi740	*⊿ompC⊿ompF + pOmpF-TOPO*	Derived from strain KJ740 with complementation of *ompF* cloned into pCR™2.1-TOPO™

TABLE 2 Primer design for PCR genotype validation

Name	Sequence (5' to 3')
rpoS forward	TCG CTT GAG ACT GGC CTT TCT G
rpoS reverse	CGG AAC CAG GCT TTT GCT TGA ATG
ompC forward	GCA TTT ACA TTT TGA AAC ATC TAT AGC G
ompC reverse	GGG TTG TGG TTT TTG ATC GC
ompF forward	ACA AAG AAG CTT^{H} GTG GCA GGT GTC ATA AAA AAA ACC
ompF reverse	GAA GAA TTC TAG A^{X}GA ACT GGT AAA CGA TAC CCA CAG

^{H} indicates HindIII cut site, while ^{X} indicates XbaI cut site

ThermoFisher Scientific (Cat#10787018) was also mixed with 10X Loading Buffer and H_2O and was used to determine the size of the DNA products. The gel ran at 120 V for 1 hour in 1X TAE buffer, and gels were visualized by the BioRad ChemiDoc™ Imaging System (Cat#12003153).

Cloning *ompF* into vector backbone. A list of considered plasmids are described in Table 3. The initial vector considered as a backbone for *ompF* was *pIG01NK*. A plate with *pIG01NK* transformed into competent DH5a cells was provided by G. Ifill of the Fernandez Lab at UBC. A miniprep was performed using the PureLink™ Quick Plasmid Miniprep Kit by ThermoFisher Scientific (Cat# K210010) for plasmid extraction. Digestion reactions were set up for both *ompF* and *pIG01NK*. Per 50uL reaction, 1 ug DNA, 1 uL of HindIII and XbaI (New England BioLabs), 10X buffer, and H_2O were added. The digestion reactions were then incubated at 37^{o}C for 1 hour. Ligation of *ompF* to *pIG01NK* was set up at a molar ratio of 3:1 respectively. 10X ligase buffer and T4 DNA ligase (ThermoFisher Scientific, Cat#15224017) were added before distilled H_2O was used to achieve a final volume of 10 uL and incubated overnight at 16^{o}C.

TOPO-TA cloning. 5' A overhangs were generated by platinum Taq and the TOPO™ TA Cloning™ Kit (ThermoFisher Scientific, Cat# K450002) was used to clone *ompF* into pCR™2.1-TOPO**™** to generate *pOmpF-TOPO* (Supplementary Fig. 1). *pOmpF-TOPO* was then transformed into One Shot® TOP10 chemically competent *E.coli*. Transformants were grown overnight on LB and LB ampicillin plates containing 5-Bromo-4-Chloro-3-Indolyl β-D-Galactopyranoside (X-Gal). Blue/white colony screening was conducted for selection of recombinants.

DNA sequencing. The *ompF* PCR reaction was purified using the PureLink™ PCR Purification Kit by ThermoFisher Scientific (Cat#K310001). The purified product and constructed plasmids were sent for sequencing at Genewiz. *ompF* was sequenced using the *ompF* forward primer, while *pIG01NK* was sequenced using the universal primer M13 forward. *pOmpF-TOPO* was isolated and sent for sequencing using the universal primers M13 forward and reverse to determine insert orientation. Sequences were aligned against the *ompF* insert sequence and NCBI database using BLAST. Upon determination of the correct

TABLE 3 Plasmid vectors considered for *ompF* cloning

Name	Feature Descriptions
pIG01NK	Spectinomycin resistance, IPTG inducible Tac promoter, C-terminal His tag
pCR2.1^{TM}TOPO^{TM}	Ampicillin resistance, Kanamycin resistance, TOPO-TA cloning, Blue/white screening

insert sequence, the ExPASy translate tool was used to determine the protein sequence and was subsequently aligned with protein BLAST.

Chemical transformation of *E. coli* strain KJ740. *E. coli* strain KJ740 cells were made competent and transformed according to the Hancock Laboratory Procedure (15). Cells were grown to log phase (0.2 to 0.4 OD_{550}) and then made chemically competent with calcium chloride. Cells transformed with *ompF* in *pIG01NK* were spread on LB agar plates supplemented with spectinomycin at 75 ug/mL and 20 mM glucose, while cells transformed with *pOmpF-TOPO* were grown with ampicillin selection and X-gal. Plates were incubated at 37°C overnight.

SDS-EDTA sensitivity assay. The protocol was adapted from Hartstein *et al* (2016). All strains were added in triplicate per EDTA concentration in 96 well polystyrene plates, and the SDS concentration was kept constant at 0.0125%. The EDTA concentrations tested were 0.02 mM, 0.05 mM, 0.1 mM, 0.15 mM, 0.20 mM, 0.25 mM, 0.3 mM, 0.35 mM, 0.40 mM, and 0.45 mM. Strains were grown overnight and OD_{600} measurements were taken to calculate cell density. Each well contained 2.7 x 10^5 cells in a final volume of 200 uL. Four strains (listed in Table 1) were used to perform this study. The plates were incubated at 37°C for 20 hours, then the OD_{600} of each well was measured using the Epoch™ Microplate Spectrophotometer by Biotek® to assess for growth.

Growth assay. All strains were incubated overnight in LB. The next day, each strain was diluted to an OD_{600} reading of 0.02 in 20 mL to start the assay. The cell suspensions were incubated at 37°C while shaking. OD_{600} measurements were taken in 30 minute intervals for 8 hours to generate a growth curve.

RESULTS

Genotype validation of *E. coli* strains. Prior to use in our assays, we needed to validate whether the provided strains were truly the correct strains. Colony PCR was performed to confirm strain identities using primers against *rpoS*, *ompC*, and *ompF*. *rpoS* was chosen as a positive control because all *E.coli* strains would express *rpoS*. Since we were using the same *E. coli* strains as Hartstein *et al.*, we also used the same *rpoS* and *ompC* primers in order to

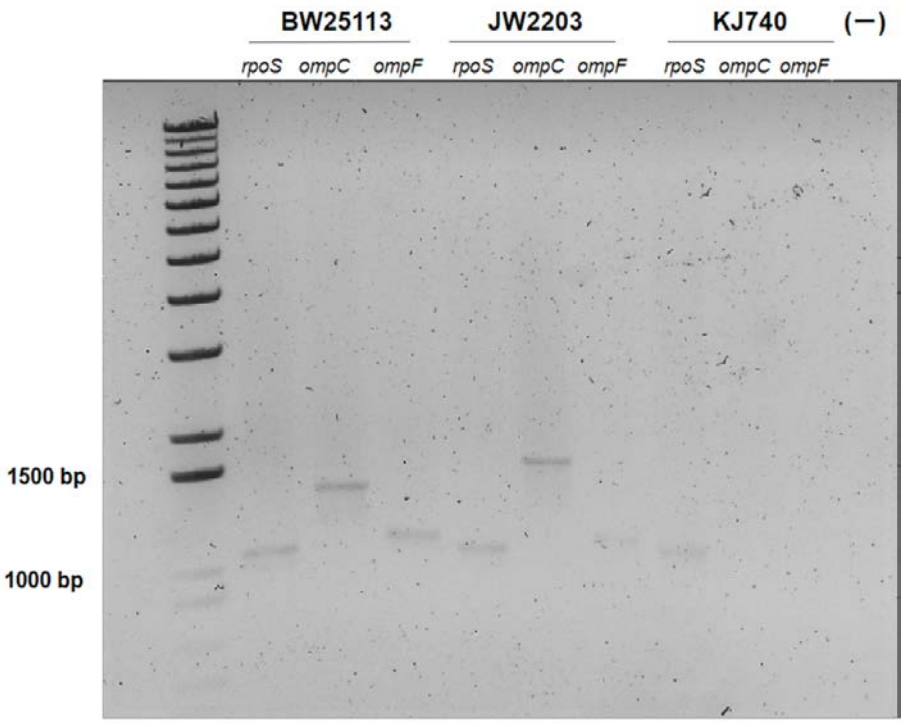

FIG. 1 Genotype validation of WT (BW25113), *ΔompC* (JW2203), and *ΔompCΔompF* (KJ740) *E. coli* strains. *rpoS*, *ompC* and *ompF* PCR products were run on 1.0% agarose gel. Amplification of *rpoS* was used as the positive control. (−) is the negative control.

more easily match strain identities. The *E. coli* strains used for validation were BW25113, JW2203, and KJ740.

Gel electrophoresis was performed to determine amplicon presence and size for genotype validation. The expected amplicon sizes of *rpoS* and *ompF* were approximately 1.1 kb (U00096.3). A 1.5 kb *ompC* product was expected for BW25113 and 1.7 kb was expected for JW2203 (7). As seen in Figure 1, the WT strain BW25113 had a distinct PCR product for each gene, confirming the presence of *rpoS*, *ompC*, and *ompF*. The *rpoS* PCR product had a size just above 1.0 kb, while the *ompC* and *ompF* PCR products had approximate sizes just below 1.5 kb and 1.1 kb respectively.

As strain JW2203 had a Kan^R cassette introduced into *ompC*, a 1.7 kb band size was expected. Again, the visualized amplicons matched the expected profile, with an *ompC* product larger than 1.5 kb and a visible *ompF* PCR product larger than 1.0 kb. Strain KJ740 was expected to not have any visible *ompC/ompF* PCR products. As seen in Figure 1, the only DNA product detected after visualization was a 1.0 kb band size corresponding to *rpoS.* Altogether, the PCR amplification of *ompF* and *ompC* strongly suggested not only did we receive the correct strains, but the strains retained the same genotype as last previously characterized (7). The *ompF* PCR products were then purified, sequenced, and aligned in preparation for plasmid construction. The alignment of our *ompF* amplicon and the NCBI *ompF* sequence resulted in a 99% sequence identity, suggesting the purified gene is indeed the WT *ompF*.

Unsuccessful generation of *ompF* containing plasmid in pIG01NK vector. Generation of an *ompF* containing plasmid was required prior to transformation of *E. coli* strain KJ740. As described in Table 3, *pIG01NK* was originally selected due to the presence of an inducible promoter which allowed for gene regulation. The *ompF* PCR product and *pIG01NK* vector were digested and agarose gel electrophoresis was conducted to validate undigested and digested *pIG01NK* vector and *ompF* insert. As shown in Supplemental Figure 2, a band size greater than 15 kb was visualized for undigested *pIG01NK*, which did not correspond to the actual *pIG01NK* size of approximately 8.0 kb. Smeared bands were observed in the

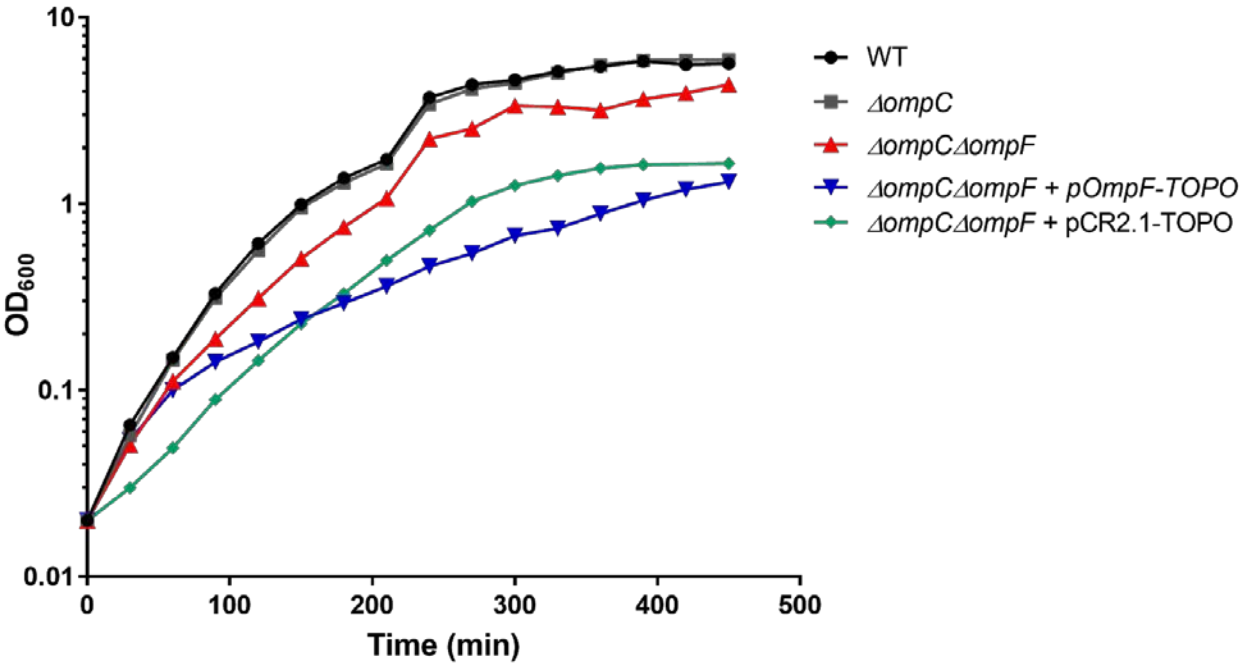

FIG. 2 Transformation of plasmid reduces growth rate. OD_{600} measurements were taken of WT, *ΔompC*-mutants, *ΔompCΔompF*-mutants, and generated *ΔompCΔompF + pOmpF-TOPO* (CyFi740) grown in LB media. An empty vector control (*ΔompCΔompF + pCR2.1-TOPO*) was added for comparison (n=1).

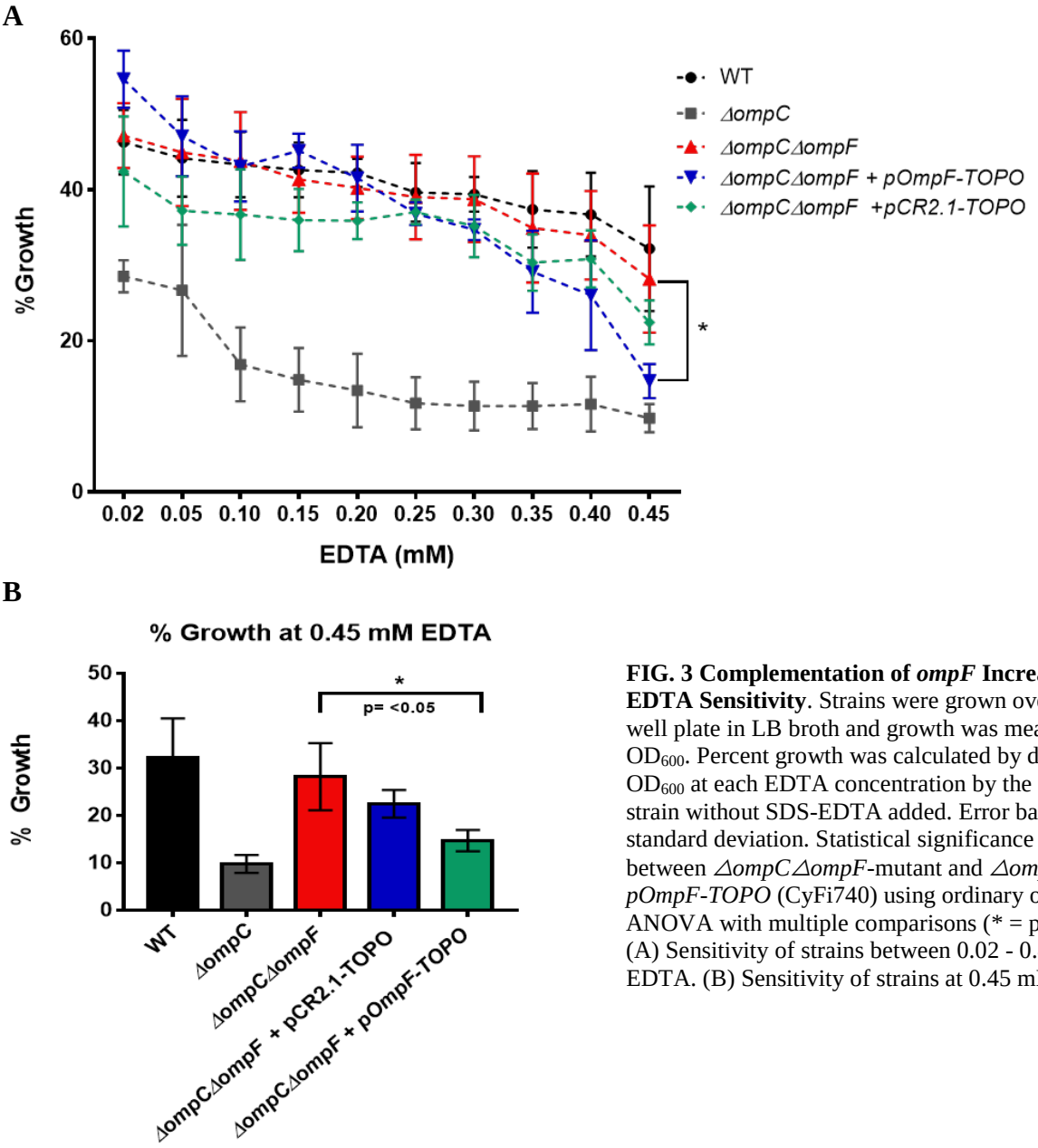

FIG. 3 Complementation of *ompF* Increases SDS-EDTA Sensitivity. Strains were grown overnight in 96-well plate in LB broth and growth was measured at OD_{600}. Percent growth was calculated by dividing the OD_{600} at each EDTA concentration by the growth of each strain without SDS-EDTA added. Error bars indicate the standard deviation. Statistical significance was calculated between *ΔompCΔompF*-mutant and *ΔompCΔompF + pOmpF-TOPO* (CyFi740) using ordinary one-way ANOVA with multiple comparisons (* = $p < 0.05$, n=3). (A) Sensitivity of strains between 0.02 - 0.45 mM EDTA. (B) Sensitivity of strains at 0.45 mM EDTA.

undigested lane, however the digested *pIG01NK* vector was not observed. Digested and undigested *ompF* produced bands at approximately 1.1 kb, which corresponded to the *ompF* insert size. Ligation of *ompF* into digested *pIG01NK* was unsuccessful in generating transformants in both DH5α and competent KJ740 cells.

Successful transformation of *ompF* into *ΔompCΔompF E.coli.* Due to the inability to generate an *ompF* containing plasmid using *pIG01NK*, attempts to create a basic vector containing our *ompF* insert was further done on pCR™2.1-TOPO™. Our rationale for selecting this vector was that Platinum Taq generated a 5' A overhang that allowed for TOPO-TA cloning. The digested *ompF* product was then cloned into the plasmid through TOPO-TA cloning. Transformation into One Shot® TOP10 chemically competent cells yielded white colonies after blue/white colony screening using X-Gal. These colonies were subsequently sent for sequencing and resulting sequences were aligned to the *ompF* gene. Sequence

analysis determined one plasmid containing the appropriate *ompF* insert orientation relative to the plasmid promoter (Supplemental Table 1, Supplemental Fig. 3, 4). M13 forward and reverse primers were used to sequence the entirety of the *ompF* insert in the plasmid. Chemically competent KJ740 cell were transformed with the constructed *pOmpF-TOPO* plasmid to generate *ompF*-KJ740 cells, designated as CyFi740. The successful generation of CyFi740 prompted investigation as to whether the transformed *E. coli* had differences in sensitivity to SDS-EDTA.

Vector transformation results in decreased growth rate of *⍙ompC⍙ompF*-mutant. After successful transformation of *pOmpF-TOPO* into KJ740 to generate *⍙ompC⍙ompF* + *pOmpF-TOPO*-mutants (CyFi740), we wanted to determine whether complementation of *ompF* hinders the growth of *⍙ompC⍙ompF E. coli.* The growth rate of the WT and *⍙ompC*-mutant were identical (Fig. 2), while there was a slight decrease in the growth rate of *⍙ompC⍙ompF*-mutants in comparison to WT. CyFi740 was observed to have an even lower growth rate, with an approximate 5-fold difference in OD_{600} when compared to WT.

The growth curve also indicated CyFi740 grew at a slower pace than *⍙ompC⍙ompF*-mutants and the WT control. However, the empty vector control (pCR™2.1-TOPO™) also exhibited a similar growth rate to CyFi740. Therefore, the decreased growth rate that was observed may be attributed to the transformation process rather than *ompF* expression.

Complementation of *ompF* into the *⍙ompC⍙ompF*-mutant decreases cell viability under increasing EDTA concentrations. The role of *ompF* in sensitivity to treatment with SDS-EDTA was determined using our SDS-EDTA sensitivity assay, as described in methods. CyFi740 exhibited a trend towards increased sensitivity compared to the *⍙ompC⍙ompF*-mutant between 0.25-0.40 mM EDTA (Fig. 3A) and was significantly ($p<0.05$) more sensitive than the *⍙ompC⍙ompF*-mutant at 0.45 mM EDTA (Fig. 3B). As expected, the *⍙ompC*-mutant was indeed more sensitive to SDS-EDTA treatment than the WT and *⍙ompC⍙ompF*-mutant (Fig. 3A). The empty vector control did not differ significantly compared to the *⍙ompC⍙ompF*-mutant. There was no growth in the negative control wells, suggesting that observed trends were unlikely due to contamination. The highest percent survival across strains ranged from 50-60%. Therefore, we determined that *ompF* complementation into *⍙ompC⍙ompF* mutants resulted in increased SDS-EDTA sensitivity.

DISCUSSION

Outer membrane porins, OmpC and OmpF, confer membrane resistance against detergents (7, 8). Deletions of these porins showed an altered resistance phenotype after exposure to SDS-EDTA. Hartstein *et al.* (2016) have established *ompC* deletion in *E.coli* confers increased sensitivity to SDS-EDTA while *ompC/ompF* double deletions did not. Atif et al. (2017) hypothesized that *ompF* single deletion mutants would exhibit sensitivity to SDS-EDTA, but found their *⍙ompF* E. coli strain showed no increased sensitivity. Based on these studies, we hypothesize OmpF expression in the absence of OmpC would result in increased sensitivity of *E. coli* to SDS-EDTA, and complementation of *ompF* back into an *⍙ompC⍙ompF* mutant will restore sensitivity to SDS-EDTA treatment.

In our project, we propose a novel model between OmpC and OmpF expression in *E. coli* towards membrane integrity (Fig. 4). Previous studies have shown OmpF has higher permeability compared to OmpC due to differences in charged residues inside the pore lumen (16). Meanwhile, Harder *et al.* (1981) has demonstrated OmpF expression may be intrinsically susceptible to anionic based disruptors, such as carbenicillin. In our model, OmpF expression in the absence of OmpC is susceptible to anionic insult due to its increased permeability. Therefore, OmpF expression would sensitize *E. coli* to anionic compounds such as SDS-EDTA.

We propose OmpC and OmpF expression have opposing effects on membrane stability in the presence of anionic agents. OmpC positively contributes to membrane stability due to

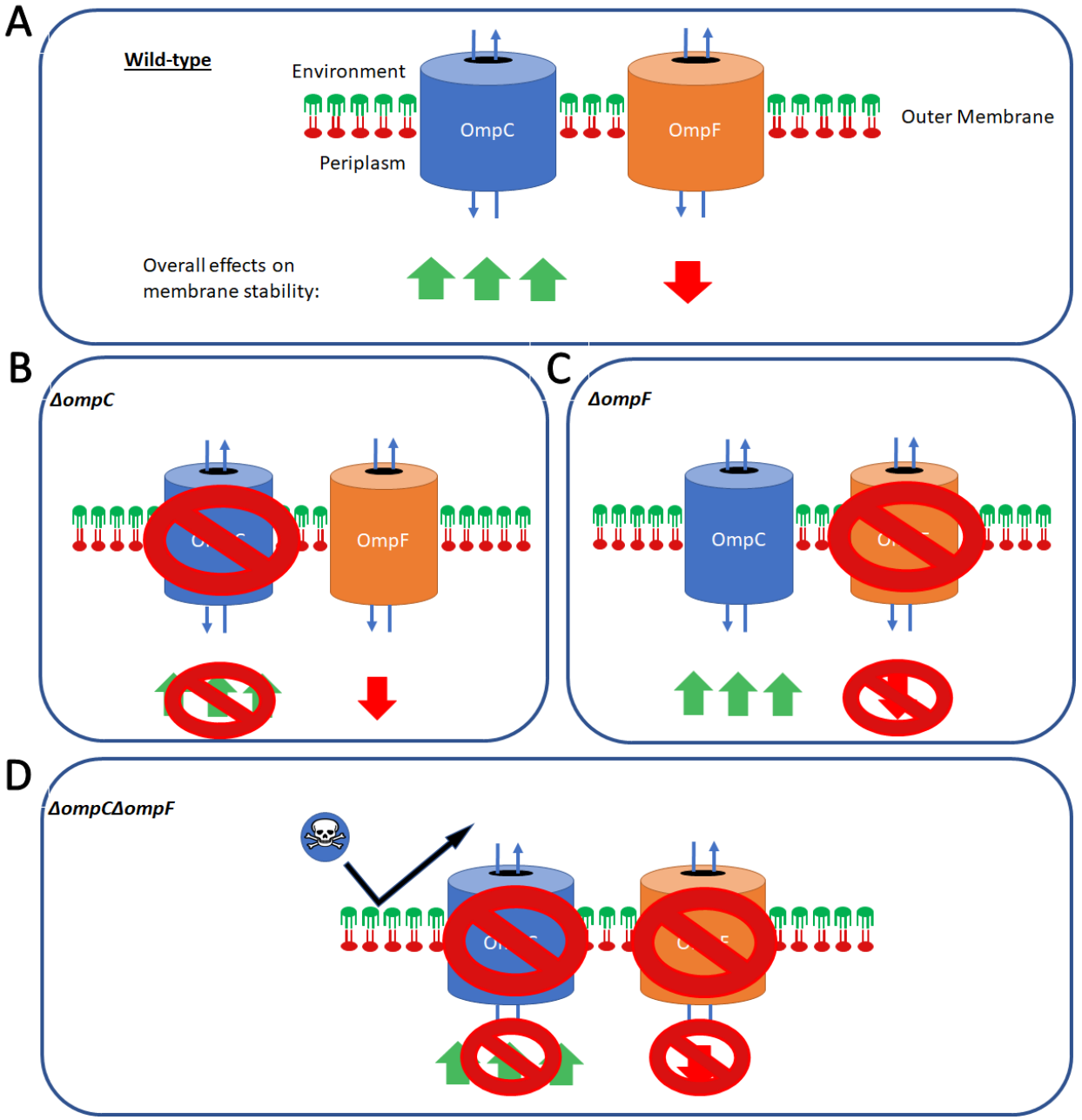

FIG. 4 Proposed model of action of OmpC and OmpF on membrane stability. (A) The presence of OmpC ameliorates the negative impact of OmpF on membrane stability. (B) Without OmpC expression, OmpF decreases membrane stability. (C) OmpF knockout does not significantly change membrane stability and (D) deletion of both results in inability of harmful molecules to infiltrate the periplasm.

its interaction with the Mla pathway (4), whereas OmpF negatively contributes to membrane stability as previously stated. In WT *E. coli* (Fig. 4A), we hypothesize the contribution of OmpC outweighs the negative effects of OmpF. In *ompC* deletion mutants (Fig. 4B), OmpF expression in the absence of OmpC results in the destabilization of the OM. However, absence of OmpF (Fig. 4C) does not result in change of membrane stability due to the stabilizing effect of OmpC. From our SDS-EDTA assays, we observe that the *ΔompC E. coli* strain JW2203 is more sensitive compared to the WT strain. Atif *et al.* (2017) have also shown deletion of *ompF* is not sufficient to confer sensitivity to SDS-EDTA, in accordance to our model. We demonstrated that *ΔompCΔompF* mutants (strain KJ740) show resistance to

SDS-EDTA similar to WT. We suggest in the absence of OmpC and OmpF, there are decreased entry points for harmful molecules into the periplasm (Fig. 4D).

Based on our model, complementation of *ompF* into *⊿ompC⊿ompF* will result in sensitivity comparable to *⊿ompC* deletion mutants. Our results show CyFi740 as significantly ($p<0.05$) more sensitive than *⊿ompC⊿ompF* at 0.45 mM EDTA. This supports our model that OmpF expression destabilizes the membrane in the absence of OmpC.

To investigate the possibility that uncontrolled expression of *ompF* negatively impacts growth, a growth assay was conducted comparing CyFi740 and the empty vector control against strain *⊿ompC⊿ompF*. Both CyFi740 and the empty vector control exhibited similar levels of diminished growth compared to *⊿ompC⊿ompF*. This suggests the decreased growth is primarily due to plasmid transformation, rather than the unregulated expression of *ompF*. In our SDS-EDTA assays, empty vector control showed no significant difference in sensitivity compared to *⊿ompC⊿ompF*. Therefore, this provides evidence that the observed sensitivity of CyFi740 is due to the presence of *ompF*. However, OmpF protein expression was not directly measured and should be the subject of further studies. In conclusion, complementation of *ompF* into *⊿ompC⊿ompF E. coli* confers increased membrane sensitivity to SDS-EDTA treatment.

Study limitations. One limitation of our study is the inability to regulate *ompF* expression level. We initially chose *pIG01NK* as our primary vector due to the presence of an IPTG inducible promoter. As *pOmpF-TOPO* has constitutive expression, it is unknown how uncontrolled *ompF* expression can affect *E. coli* sensitivity to SDS-EDTA. Another limitation of our study is that we did not confirm translation and OM expression of the OmpF protein on CyFi740.

Changes in OmpC/OmpF expression may cause regulatory effects on other OM components which our model does not account for. Additional research is required to connect protein expression with gene regulation to establish an all-encompassing model.

Future Directions Looking forward, much of the dynamic relationship between OmpF, OmpC and membrane integrity have yet to be elucidated. While our results indicate a promising trend supporting our hypothesis, more evidence is needed to establish the relationship between OmpF and membrane stability.

A potential future project would be to design an *ompF* containing plasmid with an inducible promoter, which would allow us to assess the effect of varying *ompF* expression levels. Using this method, we can confirm the trends seen here by modulating expression to match wild-type. Another study that could be used to validate our results would be to assess OmpF expression using proteomic methods such as SDS-PAGE, Western Blot, or Sarkosyl extraction. This would confirm complementation of *ompF* results in proper protein expression of OmpF on the OM.

To address the limitations of our model, transcriptomic analysis using RNA-Seq would elucidate the changes in transcription of different genes due to the presence or absence of OmpC and OmpF. This would allow for a better understanding of the interactions between porin proteins and other pathways contributing to membrane stability.

Another issue to explore is how different types of detergents could affect the outcome of the sensitivity assays. Previous studies regarding porins have implicated OmpC and OmpF to dilate and relax based on the pH and charge of the surrounding environment (18). It would be informative to explore if detergents with different charges, composition, and mode of action would change the phenotype of resistance in *E. coli* when *ompC* or *ompF* expression is perturbed.

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