

Investigating the Sex Life of *Escherichia coli*: The Effect of OmpA Expression on Hfr Conjugation Efficiency

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Bacterial conjugation is the process in which genetic material is horizontally transferred between bacteria. OmpA, a highly abundant protein in the outer membrane of *Escherichia coli*, is a critical component required for conjugation. In order to investigate the effect of OmpA expression on Hfr conjugation, the conjugation efficiencies of *E. coli* strains expressing varying degrees of functional OmpA were compared. *E. coli* wild-type (C149), OmpA⁻ (C156), and OmpA⁻OmpC⁻ (C158) served as recipient strains for the Hfr conjugation with the *E. coli* HfrC donor strain. In addition to comparing the conjugation efficiencies of parental recipient strains, the conjugation efficiencies of *E. coli* C149, C156, and C158 strains transformed with the pCCK06-1 plasmid, previously constructed to contain the *ompA* gene, were also compared. No differences in conjugation efficiencies were observed between transformed and untransformed recipient strains. Both untransformed C149 and transformed C149 (pCCK06-1) recipient strains had approximately 10-to-20-fold more successful conjugations following mating with HfrC compared to the pCCK06-1 transformed and untransformed C156 and C158 recipient strains. These results suggest that OmpA⁻ strains, C156 and C158, are able to participate in Hfr conjugations; however, these conjugations are not as efficient as wild-type (C149) strains. Introduction of plasmid-expressed functional *ompA* did not improve the conjugation efficiencies of the respective parental strains.

Horizontal transfer of genetic material within and between different bacterial species may occur by a conjugation process that involves direct cell-to-cell contact, mating-pair formation, and DNA transfer. This process is significant to the horizontal transfer of genes involved in virulence and antimicrobial resistance. Conjugation is generally mediated by conjugative pili as well as TraN and TraG on the donor cell which interact with OmpA and LPS on the recipient cell enabling mating pair stabilization and transfer of DNA (3,10). OmpA is a two-domain β -barrelled outer membrane protein. Its N terminal domain anchors in the membrane and the C terminal domain, located in the periplasmic space, interacts specifically with the peptidoglycan layer. OmpA is a highly abundant surface protein in *Escherichia coli* and is often a receptor targeted by bacteriophages and bacteriocins (4). OmpA is critical component for conjugation as strains with knockouts or mutations of OmpA protein are generally deficient as conjugation recipients. While there is some evidence of OmpA independent conjugation pathways (6), the presence of OmpA is required for efficient F conjugation (5).

While OmpA has been established as an essential component for conjugation, there has been little research on the effect of varying levels of OmpA on conjugation efficiency. For this study, C149 (wild-type), C156 (OmpA⁻) and C158 (OmpA⁻ OmpC⁻) recipient *E. coli* cells were transformed by

electroporation with the plasmid pCCK06-1, a pCR2.1-TOPO plasmid containing a cloned *ompA* gene (1). The pCCK06-1 plasmid contains a P_{lac} promoter allowing IPTG induced expression of downstream genes including *ompA*. C149, C156 and C158 recipient strains all contain the streptomycin resistance gene, *rpsL-97*, and were selected for on streptomycin-containing media. The *E. coli* HfrC (*proC*⁺) was used as a donor strain due to the unavailability of an F⁺ strain that would enable verification of conjugation in the recipient cell lines (*proC*⁺). Conjugated recipient cells with the *proC*⁺ gene from the Hfr strain were selected on media lacking proline supplementation. We tested the effect of increased or restored *ompA* expression on conjugation efficiencies between the HfrC donor strain and untransformed or pCCK06-1 transformed recipient cell lines. We observed that the conjugation efficiency was poor in the parental C156 (OmpA⁻) and C158 (OmpA⁻OmpC⁻) strains compared to the C149 (wild-type) strain and that the induction of the *ompA* gene on the pCCK06-1 plasmid had no significant effect on the conjugation frequencies.

MATERIALS AND METHODS

Bacterial Strains. All *E. coli* strains used in this experiment were obtained from the MICB 421 bacterial strain collection in the Department of Microbiology and Immunology at the University of British Columbia. *E. coli* TOP10 containing the pCCK06-1 plasmid (pCR2.1 TOPO-*ompA*) preparation was described

previously (1). *E. coli* wild-type (C149), OmpA⁻ (C156), and OmpA⁺OmpC⁻ (C158) strains with or without the pCCK06-1 plasmid served as recipient strains for the Hfr conjugation (11); the original strain JF568 was previously described (9). *E. coli* HfrC strain served as the donor strain for the Hfr conjugation. Refer to Table 1 for the complete list of strains used for the Hfr conjugation and their genotypes. *E. coli* strains C149, C156, and C158 containing the pCCK06-1 plasmid were prepared and stored in the UBC bacterial strain collection under designations CCFQ071, CCFQ072, and CCFQ073, respectively.

TABLE 1. List of *E. coli* strains used for Hfr conjugation.

Strain	Phenotype	Genotype
C149	Wild-type	F <i>prcC-24 avoA-357 his-53 purE-41 ihv-277 met-65 lacY-29 xyl-14 rpsL-97 cyaA-1 cysB-2? tsx-63 lambda</i>
C156	OmpA ⁺	F <i>prcC-24 ompA-256 his-53 purE-41 ihv-277 met-65 lacY-29 xyl-14 rpsL-97 cyaA-1 cysB-2? tsx-63 lambda</i>
C158	OmpA ⁺ OmpC ⁻	F <i>prcC-24 ompA-252 his-53 ompC-262 purE-41 ihv-277 met-65 lacY-29 xyl-14 rpsL-97 cyaA-1 cysB-2? tsx-63 lambda</i>
HfrC	Hfr	HfrPO 9680' CCW <i>metB1 rel-1</i> λ

Culture Conditions. *E. coli* TOP10-pCCK06-1 construct strain (1) was grown in Luria-Burtani (LB) broth (1% (w/v) tryptone, 0.5% (w/v) yeast extract, 85.6 mM NaCl) containing 100 µg/mL ampicillin (St. Louis, Mo., USA, Cat. No. A9518-25G). *E. coli* C149, C156, C158, and HfrC strains were grown in LB broth containing 100 µg/mL streptomycin (Sigma, Cat. No. S6501-50G) and 280 µg/mL IPTG to induce *ompA* expression from the pCCK06-1 plasmid. Following the bacterial conjugation, donor-recipient cell mixtures were plated on selective M9 minimal media (7.8 mM NaCl, 44.8 mM Na₂HPO₄, 20.0 mM KH₂PO₄, 17.0 mM NH₄Cl, 0.7 mM MgSO₄·7H₂O, pH 7.2) containing 0.4% glycerol, amino acids histidine, isoleucine, valine, methionine, tyrosine, and phenylalanine at 40 µg/mL each, 40 µg/mL adenine, 2 µM *p*-aminobenzoic acid, 2 µM *p*-hydroxybenzoic acid, 0.1 µg/mL thiamine, 0.01 µg/mL pyridoxine, and 100 µg/mL streptomycin. Selective M9 minimal media was prepared without proline supplementation, which served as the selective marker for Hfr conjugation. All supplements used in this experiment were ACS grade quality. Original culture conditions were previously described (9).

Transformation. Electrocompetent cells of C149, C145, and C158 strains were prepared according to the procedure in the Micropulser Electroporation Apparatus Operating Instructions and Application Guide (BioRad, Hercules, CA). Overnight cultures of C149, C156, and C158 were diluted approximately 1:4 in LB broth. Cultures were grown at 37°C for 30 minutes until an OD₆₀₀ reading of 0.800 was obtained. Cultures were centrifuged on a Beckman Model J2-21 centrifuge (Beckman Coulter, Inc., Fullerton, CA) using a JA-20 rotor at 5000 x g for 15 minutes at 4°C and resuspended in sequentially decreasing volumes (500 mL, 250 mL, 20 mL, and 2 mL) of an ice cold 10% (w/v) glycerol solution. Cultures were maintained on ice throughout the procedure and stored at -80°C following electrocompetent preparation. The pCCK06-1 plasmid was transformed into electrocompetent C149, C156, and C158 strains according to procedure in the Micropulser Electroporation Apparatus Operating Instructions and Application Guide. Briefly, 40 µL of electrocompetent cultures were combined with 2 µL plasmid DNA,

electroporated on a BioRad Micropulser at 2.5 kV for a single pulse on the Ec2 setting, resuspended in 1 mL LB, and incubated at 37°C for 1 hour. Following the 1 hour incubation, cultures were plated on LB-agar containing 100 µg/mL ampicillin in order to select for transformed cells.

Plasmid Isolation. The pCCK06-1 plasmid was extracted from *E. coli* TOP10 cells using the PureLink HQ Mini Plasmid Purification Kit (Invitrogen, Carlsbad, CA, Cat. No. K2100-01) according to the manufacturer's instructions. DNA quality and concentration were assessed at 260 nm and 280 nm wavelengths on a Beckman DU530 Life Science UV/Vis Spectrophotometer (Beckman Coulter, Inc., Fullerton, CA).

Restriction Digest. The pCCK06-1 plasmid was digested with restriction enzyme *EcoRI* (Gibco BRL Life Technologies, Carlsbad, CA, Cat. No. 15202-013) in a mixture containing 4 µL plasmid DNA, 2 µL 10x React 3 buffer, 1 µL *EcoRI* enzyme, and 13 µL dH₂O. Digested plasmids were run on a 0.7% non-denaturing agarose (BioRad, Hercules, CA, Cat. No. 161-3102EDU) gel in 1x TAE buffer (40.0 mM Tris Base, 0.1% (v/v) glacial acetic acid, 1.3 mM EDTA, pH 8) for 1 hour at 100 volts. Gels were stained with 0.2 µg/mL ethidium bromide and photographed using AlphaImager Software v. 4.1.0 (Alpha Innotech Corp., San Leandro, CA).

Hfr Conjugation. The conjugation procedure was previously described (8). Briefly, an overnight culture of donor strain HfrC was diluted 1:25 in LB broth and incubated at 37°C for 3 hours. Overnight cultures of recipient strains C149, C156, and C158 both with and without the pCCK06-1 plasmid were diluted 1:5 and incubated at 37°C with mild aeration for 3 hours. Following 3 hours of incubation, donor and recipient cultures were plated at 10⁻¹, 10⁻², 10⁻³ dilutions on LB-agar in order to assess baseline cell density. Donor and recipient strains were combined in a 1:1 ratio (0.2 mL of each culture) and incubated at 37°C for 1.5 hours without aeration. Following the conjugation, 2 mL of LB broth was added to each donor-recipient pair culture and grown at 37°C with mild aeration for an additional 1.5 hours. Cultures were plated at 10⁻¹, 10⁻², 10⁻³ dilutions on selective M9 minimal media containing 100 µg/mL streptomycin and supplements as described above without the addition of proline, which was used as the selective marker for the conjugation. Plates were incubated at 37°C for 48 hours. Non-conjugation controls were prepared as above; however, only the recipient strain (0.2 mL) without the donor strain was used for the conjugation.

RESULTS

Plasmid Isolation and Restriction Digest. The pCCK06-1 plasmid was successfully isolated from the previously transformed *E. coli* TOP10 strain (1). *EcoRI* digest of isolated pCCK06-1 plasmid produced three bands at approximately 5.1 kb, 3.9 kb, and 1.2 kb representing partially digested pCCK06-1 plasmid, pCR2.1 TOPO vector, and ligated *ompA* PCR product, respectively, when separated on a non-denaturing agarose gel (Fig. 1). The band sizes are consistent with those expected from the pCCK06-1 plasmid described by Chan *et al.* (1). The pCCK06-1 plasmid isolated from *E. coli* TOP10 was subsequently transformed into electrocompetent *E. coli* C149, C156, and C158 strains and plated onto selective LB-ampicillin plates. The *EcoRI* digest of the pCCK06-1 plasmid isolated from transformed C149, C156, and C158 strains produced two bands at approximately 3.9 kb and 1.2 kb representing the

pCR2.1 TOPO vector and ligated *ompA* PCR product, respectively, when separated on a non-denaturing agarose gel (Fig. 2).

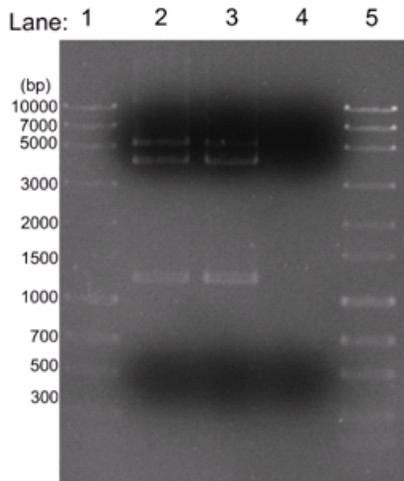


FIG. 1. Restriction enzyme digestion of pCCK06-1 plasmid DNA isolated from TOP10 *E. coli*. Lanes 1,5: MassRuler™ DNA ladder (Fermentas, Burlington, ON, Cat. No. SM1283), Lanes 2-3: *EcoRI* digested pCCK06-1 plasmid DNA. Lane 4: Blank.

Hfr Conjugation. Baseline cell densities of the cultures on non-selective medium prior to the conjugation were unable to be quantified due to confluent growth on the plates. No growth was observed on the non-conjugated control samples plated on selective media, which suggests that conjugation of genes from the HfrC strain were required for growth of the recipient strains on supplemented M9 minimal media lacking proline and that the HfrC donor strain was streptomycin sensitive. No conjugation efficiency differences were observed between the recipient strains with or without the pCCK06-1 plasmid (Table 2). An approximately 10-to-20-fold increase in cell density was observed, however, for the C149 parental and C149 (pCCK06-1) strains in comparison to the C156 and C158 parental and pCCK06-1-containing strains (Table 2). The majority of plates at the 10^{-2} dilution and all of the plates at the 10^{-1} dilution produced colonies that were too numerous to count; therefore, these results only approximate the conjugation efficiencies of each recipient strain. Because the baseline cell densities were not quantifiable, conjugation efficiency in this context refers to the number of viable cells following Hfr conjugation. This definition assumes that all recipient strains were conjugated with the HfrC donor strain in approximately equal cell densities. Because all recipient strains were incubated under the same

conditions and diluted at the same ratio, this assumption is presumably accurate.

DISCUSSION

E. coli strains C156 and C158 were selected for testing conjugation efficiency due to their *OmpA* phenotype. Strains C156 and C158 that lacked functional *OmpA* had an approximately 10-to-20-fold lower conjugation efficiency compared to the C149 strain, which was consistent with our hypothesis that *OmpA* mutants are generally deficient as conjugation recipients.

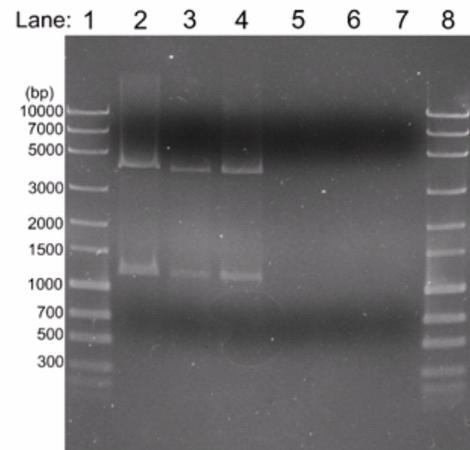


FIG. 2. Restriction enzyme digestion (*EcoRI*) of pCCK06-1 plasmid DNA isolated from *E. coli* C149, C156, and C158 transformants. Lanes 1,8: MassRuler™ DNA ladder (Fermentas, Burlington, ON, Cat. No. SM1283), Lane 2: C149 plasmid, Lane 3: C156 plasmid, Lane 4: C158 plasmid, Lane 5: untransformed C149, Lane 6: untransformed C156, Lane 7: untransformed C158.

TABLE 2. Recombinant concentration observed on minimal selective media following conjugation between the pCCK06-1 transformed and untransformed recipient strains C149, C156, and C158 and donor strain HfrC.

Strain	Plasmid	Recombinant Conc. (10^4 CFU/ml)
C149	None	30
	pCCK06-1	40
C156	None	5
	pCCK06-1	3
C158	None	1
	pCCK06-1	3

Recipient strains were assumed to be at similar cell concentrations during the conjugation experiments; therefore, we have inferred that the variable growth of cells on the incomplete media was a result of inherent variability in conjugation efficiencies due to strain phenotype. As noted previously, baseline cell

densities of the cultures prior to the conjugation were not quantified due to confluent bacterial growth. In addition to missing baseline data, the majority of conjugated bacterial cultures produced colonies that were too numerous to count and some contained satellite colonies. Consequently, conjugated cell concentration calculations are based on only the 10^{-2} and 10^{-3} dilution factors and are likely only approximations of the actual conjugated cell concentration. The colony counts do carry a significant error margin as only single plates were made for each dilution. There is also a possibility the increased number of colonies counted on the C149 and C149 (pCCK06-1) plates are not statistically significant, as only one of several dilution plates contained countable (30 to 300) colonies. Duplicate or triplicate plates for each dilution would have confirmed if these two strains had significantly different conjugation efficiencies. For these reasons, the calculated conjugated cell concentrations, which are being used as a proximate for conjugation efficiency, are only approximations.

Proline auxotrophy and streptomycin resistance were used as selective markers for recipient bacteria that underwent successful conjugation with an HfrC donor. Growth of C149, C156, and C158 strains on minimal selective medium supplemented with proline was not attempted due to last minute alterations of the supplement mixture and time constraints of the experiment; therefore, unknown selective factors in addition to proline may be present. However, based on the location of *proC* on the genome with respect to the HfrC origin of transfer, the transfer direction, and the recipient strains Pro⁻ phenotype, proline was deemed the most likely selective marker in our experiment. The HfrC origin lies at 96.80' and is replicated in a counter-clockwise direction. The *proC* gene lies at approximately 10.5' (7); thus, a high proportion of bacteria that initiated transfer would have received the *proC* gene and any preceding genes as the length of genetic material required for successful growth on the medium was relatively short. Conjugations that initiated but failed to restore the Pro⁺ phenotype in the recipients would not have grown; however, the proportion of these failed transfers would have been consistent among the six recipient strains. Streptomycin was included in the selective media to remove remaining donor cells from the final plate counts. All seven strains involved in the conjugation experiment (donor and recipients) were plated on the selective media and showed no growth, confirming that genes acquired from the donor strain allowed the recipients to grow and that the HfrC strains were streptomycin sensitive.

Previous studies have identified several functions of OmpA proteins including providing resistance to antibiotics and chelating agents, providing receptor targets to TuII and K3 bacteriophages, binding cells together during conjugation, and facilitating uptake of colicins (6). Manoil *et al.* (6) also characterized different classes (A1-A3) of conjugation-deficient OmpA mutants in which mutants may have differing susceptibilities to bacteriophages. These mutants lack detectable amounts of OmpA polypeptide (A1), have normal amounts of OmpA (A2), or have detectable but reduced amounts of OmpA protein (A3). These three classes of OmpA mutants had different relative amounts of deficient conjugation efficiency compared to an OmpA wild-type strain. Manoil *et al.*'s results also suggested that a conjugation pathway is possible in the absence of OmpA protein. An OmpA independent conjugation pathway may also be responsible for the successful conjugation of the untransformed C156 and C158 strains, which lack the *ompA*-containing plasmid (pCCK06-1). Unfortunately due to limited resources and time constraints, we were unable to quantify the OmpA membrane protein expression or gene sequences of C149, C156, and C158, which may have given a better indication of the class of OmpA mutant type. It was unclear if the OmpA mutant phenotypes were due to reduced protein expression or reduced conjugation efficiency resulting from proteins with structural mutations.

The C156 (pCCK06-1) and C158 (pCCK06-1) strains did not have improved conjugation efficiency relative to untransformed C156 and C158 strains. The presence of the pCCK06-1 plasmid was confirmed in Fig.2. It is possible that the *ompA* gene on pCCK06-1 plasmid was not being expressed, which resulted in no change in OmpA protein levels, despite IPTG induction. We did not have sufficient time to perform a membrane protein assay to quantify levels of OmpA protein and Chan *et al.* (2006) were unable to confirm the efficacy of pCCK06-1 in increasing OmpA levels in their previous work. As OmpA is a highly expressed protein with estimates of 2-3 x 10⁵ copies per cell (2), we speculate that chromosomal *ompA* has a stronger promoter which may lead to higher levels of chromosome produced OmpA protein relative to OmpA protein produced by pCCK06-1. Thus the C156 and C158 strains defective OmpA proteins expressed by chromosomal DNA may be of higher proportion to the OmpA produced by the pCCK06-1 plasmid, which would contribute to the lack of detectable difference in conjugation efficiency between C156 (pCCK06-1) and C158 (pCCK06-1) strains.

Our results indicate that wild-type C156 and C158 as well as transformed C156 (pCCK06-1) and

C158 (pCCK06-1) strains were capable of undergoing Hfr conjugation despite lacking a functional OmpA protein. These findings are significant considering that functional OmpA is typically required for successful conjugation. Our results suggest that the specific *ompA* mutation in our recipient strains did not prevent conjugation, but rather impaired its efficiency in comparison to wild-type C149 and transformed C149 (pCCK06-1) strains, which produced approximately 10-to-20-fold higher successful conjugates. Further research is required to more accurately calculate the conjugation efficiencies of the *E. coli* C149, C156, and C158 strains as well as better characterize the *ompA* mutation in these strains and determine its role in conjugation.

FUTURE EXPERIMENTS

Elucidation of expression levels is necessary in order to assess whether or not differences in conjugation efficiency (or lack thereof) resulted from actual differences in protein levels. Confirmation of expected expression levels is necessary to determine whether discrepancies between expected and observed levels of *ompA* expression might explain some of our observations. Extracting membranes and isolating membrane proteins from the relevant strains and quantifying protein levels are possible methods of quantifying *ompA* expression. Originally, we had intended to do this experiment through physical lysis of cell cultures followed by sequential enrichment of lysates for membrane proteins through centrifugation and extraction in appropriate buffers. Ideally, SDS-PAGE of the membrane extraction followed by Western blot analysis with an OmpA-specific antibody would provide an estimate of relative protein expression levels. If resources and time constraints preclude the use of an OmpA antibody, OmpA could still be detected with a Coomassie Blue stain, as OmpA constitutes a large percentage of *E. coli* membrane proteins (2). Other methods could also be used to determine expression levels such as RT-PCR. A determination of OmpA expression levels in these strains will provide critical information that will clarify our results as well as provide insight as to the direction in which this work can be progressed.

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