Supplemental Information

for

Outer Membrane Porin F (OmpF) in E. coli is Critical for Effective Predation by Bdellovibrio

Running Title: Prey-Specific Recognition by Predatory Bacteria

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Supplementary methods

Bacterial strains and culturing conditions
All the bacterial strains used in this study are listed in Table S1. Each of the prey and their isogenic mutants were routinely propagated on lysogen broth (LB) agar plates. Fresh single colonies were cultured in LB broth, incubated overnight in a shaking incubator at 37 ºC, centrifuged (5000 x g, 15 min) and the pellet was re-suspended in the predation media. All the predatory strains were routinely grown as described previously (1, 2) using E. coli MG1655/pUCDK as the prey.

Bioluminescence assay to monitor the predation kinetics
The E. coli prey strains were rendered bioluminescent by transforming them with pGEN-luxCDABE (3), a gift from Harry Mobley (Addgene plasmid # 44918; http://n2t.net/addgene:44918; RRID: Addgene_44918). Overnight cultures of these prey strains were grown in LB broth supplemented with 100 µg/ml ampicillin at 37 ºC before being diluted to an optical density (OD600nm) of 0.05 in dilute nutrient broth (DNB; 1/10 NB) containing 3 mM MgCl2 and 2 mM CaCl2. The predator was grown as described above, filtered, and diluted two-fold in 25 mM HEPES (with 3 mM MgCl2 and 2 mM CaCl2, pH 7.2). The predator and prey cell densities in each sample were determined using top agar plates and colony counts, respectively, as described previously (4), and were used to calculate the predator-prey ratio (PPR). The predator and prey preparations were mixed 1:1 (v:v; 100 µl each) in the wells of a 96-well plate (white, Greiner, USA) and the bioluminescence was measured every ten minutes as described previously (5).

Prey viability assessment
For these experiments, the prey was diluted to OD 0.05 in DNB. B. bacteriovorus 109J was grown as above and diluted in HEPES buffer (with 3 mM MgCl2 and 2 mM CaCl2, pH 7.2). The predator dilutions and prey suspensions were mixed (1:1 (v:v)) so that the predator-to-prey ratio was 6.25, 25 or 100. Each sample was then incubated with shaking incubator (250 rpm) at 30 ºC for 1 hour, after which the viability of the prey was determined using plate counts on LB agar plates.

Complementation of E. coli BW25113 ΔompF and E. coli BW25113 ΔompC
The pCA24N plasmid was linearized for In-Fusion® cloning using primers pCA ir and pCA if primers (Table S2). The ompF gene was amplified from E. coli MG1655 with its flanking
regions using primers pCA-Omp F and pCA-Omp R primers (Table S2). After purifying both
the vector and insert, they were recombined using the In-Fusion® manufacturer’s suggested
protocol, generating the complementation plasmid, pCA-ompF. This plasmid was
transformed into E. coli DH5α cells, which were then grown on LB agar plates containing
chloramphenicol (35 µg/ml). The plasmid from an individual colony was purified and
sequenced using the pCA24 seq F and pCA24 seq R primer set (Table S2). Once the
sequence was verified, the plasmid (pCA-ompF) was transformed into the E. coli BW25113
strains.

The same was used to also construct the pCA-ompC plasmid. For this, the ompC gene was
amplified from wild-type E. coli BW25113 with additional flanking region using primers
pCA-ompC F and pCA-ompC R (Table S2). After construction, transformation and
purification of the plasmid as above, its sequence was confirmed using primers pCA24 seq F
and pCA24 seq R (Table S2).

**Microscopic analyses of predation**

Using E. coli S17 λ-pir as the donor, plasmid pMQ414, which expresses the tdTomato
fluorescence protein (6), was transferred into B. bacteriovorus 109J through conjugation. The
predation tests were conducted using this fluorescent predatory strain and a synchronized
attack, to ensure many of the prey were attacked at the same time, as described previously (7).
Briefly, after growth of the fluorescent predator using the same protocol as described above,
it was concentrated 10-fold by centrifugation (7000 x g, 15 min) and resuspended in fresh
HEPES buffer (with 3 mM MgCl₂ and 2 mM CaCl₂, pH 7.2). For these predation assays,
wild-type E. coli BW25113 and three isogenic mutants (i.e., ΔompF, ΔompR and ΔenvZ)
were all used as prey. Each was grown overnight as above, centrifuged (7000 x g, 15 min)
and resuspended in HEPES buffer to an OD₆₀₀nm of 4.0. These samples (i.e., predator and
prey solutions) were stored at 30 °C for 10 minutes before they were mixed 1:1 (v:v). The
mixed cultures were incubated in a shaking incubator at 30 °C and samples were taken at set
times (i.e., 0, 20 and 60 min) and fixed with an equal volume of 8% (w/v) paraformaldehyde
(PFA) prepared in the same HEPES buffer. The fixed samples were stored at 4 °C until being
observed by confocal microscopy. The number of each prey cell type (i.e., free prey, prey
with a predator attached or bdelloplast) were counted and analyzed at the indicated time
points.
Growth of *E. coli* at Higher Osmolalities

To study the impact of the medium osmolality on predation rates, *E. coli* BW25113 and *E. coli* JW0912 (ΔompF) were grown in LB medium prepared without NaCl addition. Before autoclaving, NaCl was added to a final concentration of 0, 0.25, 0.5 and 1% (w:v), generating osmolalities of 78, 162, 256 and 427 mOsm/kg, respectively, for each medium. Growth of the prey was conducted as described above. After growth overnight, the prey cells were pelleted (5000 x g, 15 min), washed in sterile HEPES to remove the salts (1, 8) and resuspended in fresh DNB to an OD of 0.05, as described previously (9).

**P1 transduction to generate ompF knockouts in the different *E. coli* strains**

*E. coli* JW0912 (i.e., the isogenic *E. coli* BW25113 ΔompF strain from the KEIO collection (10)) was used as the donor strain for the preparation of the P1 phage lysate. The host *E. coli* strains used were *E. coli* BL21(DE3), *E. coli* MG1655 and *E. coli* DSM 613. After transduction according to the previously published protocol (11), successful mutants were positively selected using kanamycin (35 µg/ml) plates containing 5 mM sodium citrate. Deletion of the *ompF* gene in each *E. coli* strain was confirmed by PCR using the primers listed in Table S2.

**Constructing ompF knockout mutants in the non-*E. coli* prey**

Deletion of the *ompF* gene in *E. fergusonii* and *ompK35* (*ompF* homologue (68% identity based on amino acid sequence)) in *K. pneumoniae* was achieved using a suicide plasmid as described previously (12). Briefly, a suicide plasmid harboring a sacB gene cassette, kanamycin resistance gene cassette, the R6K replication origin and a RP4-oriT was constructed. Sets of primers (Table S2) were used to amplify approximate 1 kb homologous recombination arms flanking the genes in *K. pneumoniae* and *E. fergusonii*. These homologous recombination arms included the first and the last 20~30 amino acids of the gene in each case. Each was then fused through a third PCR reaction and ligated to the suicide plasmid using the In-Fusion® HD Cloning kit (Clontech). The ligated plasmid was transformed into *E. coli* S17 λ-pir through chemical transformation and transferred via conjugation to the corresponding recipient strain (*E. fergusonii* or *K. pneumoniae*). *E. fergusonii* ATCC 35473 and both *K. pneumoniae* WGLW1 and WGLW2 are naturally resistant to ampicillin, allowing us to screen the conjugants on LB agar plates containing 100 µg/mL ampicillin (to select against the *E. coli* S17 λ-pir donor cells) and 50 µg/mL kanamycin (to select for merodiploids). One merodiploid mutant in each case was selected.
and grown on an LSW-sucrose agar plate (tryptone 10 g/L, yeast extract 5 g/L, glycerol 5 ml/L, NaCl 0.4 g/l, sucrose 100 g/L and agar 20 g/L) (12) to generate a double crossover mutant. One colony was then selected and grown on LB agar with no antibiotics. Loss of the conjugated plasmid in this strain was confirmed by PCR using the primers listed in Table S2, as well as phenotypically as the mutant was unable to grow in presence of kanamycin.

To generate an ompF deletion in S. enterica LT2, lambda red recombineering was employed as previously described (10, 13). The resulting ompF knockout clone had its gene replaced with the kanamycin resistance cassette, leaving only the first 30 and last 18 amino acids of the host ompF gene.

**Isolation of novel BALO strains**

*B. bacteriovorus* strains EY2.3, EY3.2, DH1 and SM1 (Table S1) are all environmental isolates. *B. bacteriovorus* EY2.3 and EY3.2 were isolated from the Eonyang Wastewater Treatment Plant (Eonyang, South Korea), while *B. bacteriovorus* DH1 and SM1 were isolated from forest soil in Ulju-gun, Ulsan, South Korea. To isolate each, samples from these sites were gently mixed with 20 ml of HEPES (3 mM MgCl2 and 2 mM CaCl2, pH 7.2) in a benchtop mixer for an hour. After centrifuging the samples (1000 x g for 5 minutes) to settle down large particulates, the supernatants were collected, and 1 ml was mixed with 10 ml of prepared molten top agar (DNB supplemented with the salts and prey (*E. coli* MG1655)). The plates were incubated at 30 °C until clear plaques were visualized. Individual plaques were then collected and sub-cultured with freshly prepared prey to isolate the predators. Each predatory strain was identified as being *Bdellovibrio* based on their 16S rDNA sequence, which had high homology to that of *B. bacteriovorus* 109J (Table S3).

**Reproducibility and statistical analysis**

Unless specified, each experiment was performed in triplicate and the standard deviations are presented on the graphs as error bars. Normal distribution of each dataset was verified using the Shapiro-Wilk test. None of the samples showed a substantial departure from the normality (p > 0.05) and, thus, the student t-test was used to evaluate statistical significance between two sets of data. Significance is indicated within the graphs using: a - p < 0.05; b - p < 0.01; c - p < 0.001.
### Supplementary figures and tables

**Table S1.** Strains and plasmids used in this study

| Prey Bacterial Strains | Description | Gene Function | Ref |
|------------------------|-------------|---------------|-----|
| **Prey Bacterial Strains** |             |               |     |
| *E. coli* BW25113      | Wild-type strain |             | (10) |
| *E. coli* JW5195       | Isogenic ΔtonB  | Component of the energy transducing Ton system | (10) |
| *E. coli* JW5086       | Isogenic ΔfepA  | Ferric enterobactin outer membrane transporter | (10) |
| *E. coli* JW2341       | Isogenic ΔfdL   | Long-chain fatty acid outer membrane channel | (10) |
| *E. coli* JW0146       | Isogenic ΔfhuA  | Ferrichrome outer membrane transporter | (10) |
| *E. coli* JW0940       | Isogenic ΔompA  | Outer membrane protein A | (10) |
| *E. coli* JW2203       | Isogenic ΔompC  | Outer membrane protein C | (10) |
| *E. coli* JW0912       | Isogenic ΔompF  | Outer membrane protein F | (10) |
| *E. coli* JW1312       | Isogenic ΔompG  | Outer membrane protein G | (10) |
| *E. coli* JW3846       | Isogenic ΔompL  | Putative outer membrane protein L | (10) |
| *E. coli* JW1371       | Isogenic ΔompN  | Outer membrane protein N | (10) |
| *E. coli* JW3368       | Isogenic ΔompR  | EnvZ/OmpR two-component response regulator | (10) |
| *E. coli* JW0554       | Isogenic ΔompT  | OmpT family outer membrane protease | (10) |
| *E. coli* JW1248       | Isogenic ΔompW  | Outer membrane protein W | (10) |
| *E. coli* JW0799       | Isogenic ΔompX  | Outer membrane protein X | (10) |
| *E. coli* JW3367       | Isogenic ΔenvZ  | EnvZ/OmpR two-component sensor histidine kinase | (10) |
| *E. coli* MG1655       | Wild-type strain | Isogenic ΔompF | Outer membrane porin F | This study |
| *E. coli* MG1655 ΔompF | Wild-type strain | Isogenic ΔompF | Outer membrane porin F | This study |
| *E. coli* BL21(DE3)    | Wild-type strain | Isogenic ΔompF | Outer membrane porin F | This study |
| *E. coli* DSM 613      | Wild-type strain | Isogenic ΔompF | Outer membrane porin F | This study |
| *E. coli* DSM 613 ΔompF| Wild-type strain | Isogenic ΔompF | Outer membrane porin F | This study |
| *E. fergusonii* ATCC 35469 | Wild-type strain | Isogenic ΔompF | OmpF homologue | This study |
| *E. fergusonii* ATCC 35469 ΔompF | Wild-type strain | Isogenic ΔompF | OmpF homologue | This study |
| *Salmonella enterica* LT2 | Wild-type strain | Isogenic ΔompF | OmpF homologue | This study |
| *Salmonella enterica* LT2 ΔompF | Wild-type strain | Isogenic ΔompF | OmpF homologue | This study |
| *Klebsiella pneumoniae* WGLW1 | Wild-type strain | ΔompK35 | OmpF homologue | This study |
| *K. pneumoniae* WGLW1 ΔompK35 | Wild-type strain | ΔompK35 | OmpF homologue | This study |
| *Klebsiella pneumoniae* WGLW2 | Wild-type strain | ΔompK35 | OmpF homologue | This study |
| *K. pneumoniae* WGLW2 ΔompK35 | Wild-type strain | ΔompK35 | OmpF homologue | This study |
| **Predatory Bacterial Strains** | Characteristics |               |     |
| *Bdellovibrio bacteriovorus* 109J | Wild-type strain | ATCC 43826 |     |
| *Bdellovibrio bacteriovorus* HD100 | Wild-type strain | DSM 50701 |     |
| *Bdellovibrio* str. YE2.3 | Wild-type strain | New isolate from the Eonyang wastewater treatment plant | This study |
| *Bdellovibrio* str. YE3.3 | Wild-type strain | New isolate from the Eonyang wastewater treatment plant | This study |
| *Bdellovibrio* str. DH1 | Wild-type strain | New isolate from forest soil near Eonyang, South Korea | This study |
| *Bdellovibrio* str. SM1 | Wild-type strain | New isolate from forest soil near Eonyang, South Korea | This study |
| **Plasmids** | Characteristics |               |     |
| pGEN-luxCDABE          | Expresses luxCDABE; Generates bioluminescence | (3)      |
| pCA24N                 | Empty vector | (16)          |
| pCA-ompF               | Expresses ompF: complementation | This study |
| pCA-ompC               | Expresses ompC: complementation | This study |
Table S2. List of primers used in this study

| Primer      | Sequence                                      | Purpose                                      |
|-------------|-----------------------------------------------|----------------------------------------------|
| pCA ir      | ACGCAGGAAAAAGACATGTGAG                      | Constructing ompF complementation plasmid    |
| pCA ir      | GACCTGACGCAAAGCTTA                        | Constructing ompF complementation plasmid    |
| pCA-Omp F   | TCACATGGTTTCTCCTGGTGTGCTGTAATA             | Constructing ompF complementation plasmid    |
| pCA-Omp R   | ATTAAAGCTGTCGAGCTTCTAGAACCTG               | Constructing ompF complementation plasmid    |
| pCA-OmpC F  | TCACATGGTTTCTCCTGGTATCGAAGTCTG             | Constructing ompC complementation plasmid    |
| pCA-OmpC R  | ATTAAGCTGTCGAGCTTCTAAGAACCCGTG             | Constructing ompC complementation plasmid    |
| pCA24 seq F | TGGAAAAACGCGACGACAC                      | Sequence verification of ompF and ompC complementation plasmids |
| pCA24 seq R | CTGAAACAAATCCAGATGAGTCTG                  | Sequence verification of ompF and ompC complementation plasmids |
| Kn R        | TCAGAAAGACTCTGTAATAA                      | Amplifying the suicide plasmid used for knocking out ompF in E. fergusonii and K. pneumoniae |
| SacB F      | GGAATATTAGACATTGGAATTC                      | Amplifying the suicide plasmid used for knocking out ompF in E. fergusonii and K. pneumoniae |
| Ef-ompF up F | GTGTTCTTCCAGCGTGAAG                      | Cloning upstream homology arm for E. fergusonii ompF |
| Ef-ompF up R | TGGGGTTACTCTGTAACAC                      | Cloning upstream homology arm for E. fergusonii ompF |
| Ef-ompF Dn F | TGCTTCCCTGCTCTGTTGAGCACGTAGCTCC            | Cloning downstream homology arm for E. fergusonii ompF |
| Ef-ompF Dn R | CAGGCTAAGTCTGTTACGAT                      | Cloning downstream homology arm for E. fergusonii ompF |
| Kn-Ef-ompF F | TTTTCCAGAGTTTCTCTGAGTTGTTCCA             | Fusing the two homology arms for knocking out ompF in E. fergusonii |
| Sac-Ef-ompF F | TTGGCAACTGTAACAC                      | Fusing the two homology arms for knocking out ompF in E. fergusonii |
| Ef-ompF seq up F | CAGAATTATTGCTGCGAGCT                   | Sequence verification of E. fergusonii ompF   |
| Ef-ompF confirm F | AGAGAAGGACGAAAAACCT                  | Sequence verification of E. fergusonii ompF   |
| Ef-ompF confirm R | GCTTGCAGATGACGTCTCT                  | Sequence verification of E. fergusonii ompF   |
| Kp-ompF up F | GATTCGTCCTTCTGAGCAGC                    | Cloning upstream homology arm for constructing K. pneumoniae ompF |
| Kp-ompF up R | GATTCGTCCTTCTGAGCAGC                    | Cloning upstream homology arm for constructing K. pneumoniae ompF |
| Kp-ompF Dn F | TGGTACGGCGCTGACGCAACGCTGACGACCA      | Cloning downstream homology arm for constructing K. pneumoniae ompF |
| Kp-ompF Dn R | CTATCATCCAGGTGGTACCTCTTCTCTCCTCCCTG        | Cloning downstream homology arm for constructing K. pneumoniae ompF |
| Kp-ompF Dn R | TTTTCCAGAGTTTCTCTGAGTTGTTCCA             | Fusing the two homology arms for knocking out ompF in K. pneumoniae |
| Kp-ompF Dn R | GATTCGTCCTTCTGAGCAGC                    | Cloning upstream homology arm for constructing K. pneumoniae ompF |
| Kp-ompF Dn R | TTTTCCAGAGTTTCTCTGAGTTGTTCCA             | Fusing the two homology arms for knocking out ompF in K. pneumoniae |
| Kp-ompF Dn R | TTTTCCAGAGTTTCTCTGAGTTGTTCCA             | Fusing the two homology arms for knocking outompF in K. pneumoniae |
| Kp-ompF Dn R | GATTCGTCCTTCTGAGCAGC                    | Cloning upstream homology arm for constructing K. pneumoniae ompF |
| Sa-ompF-Kn F | GCAAATCTTCGAGCTGAGCTTCCAGCTGCC         | Knocking out ompF in S. enterica through lambda red recombining |
| Sa-ompF-Kn R | TCAGAATCTGTAAGTATACCGACAGCAGGG          | Knocking out ompF in S. enterica through lambda red recombining |

Table S3. Identification of the new predatory isolates

| Predatory Strain | Isolation Locale | Homology | Gene Region* |
|------------------|------------------|----------|--------------|
| *Bdellovibrio* str. EY2.3 | Eonyang WWTP | 98.92% (1378/1393 bp) | 1036992 ~ 1038386 |
| *Bdellovibrio* str. EY3.3 | Eonyang WWTP | 98.92% (1378/1393 bp) | 1036992 ~ 1038386 |
| *Bdellovibrio* str. DH1 | Forest Soil | 99.73% (1453/1457 bp) | 1036920 ~ 1038376 |
| *Bdellovibrio* str. SM1 | Forest Soil | 100% (1457/1457 bp) | 1036920 ~ 1038376 |

* Based on the published 16S rDNA gene sequence for *B. bacteriovorus* 1093 (NCBI Accession No. NZ_CP007656.1)
Figure S1. Plots showing the predation kinetics over 1.5 hours with *E. coli* BW25113 (Top) and its isogenic Δ*ompF* mutant strain, *E. coli* JW0912 (Bottom). Both strains harbored pGen-LuxCDABE (Table S1), making them bioluminescent. The results show the clear dose-dependent loss in bioluminescence according to the initial PPR value for *E. coli* BW25113, while the isogenic Δ*ompF* mutant showed almost no loss, although a PPR of 70 showed a downward trend, indicating this mutant strain is not truly resistant (Figure S2). (n = 3)
Figure S2. Although the ΔompF isogenic mutant of *E. coli* BW25113 is not predated efficiently by *B. bacteriovorus* 109J, it is not resistant. This graph shows that predation of the *E. coli* ΔompF mutant was still not very apparent (< 1-log reduction) after 16 h but, by 24 h, the prey viability was no different than that of wild-type *E. coli* BW25113, both reduced by more than 4-log. (n = 3)
Figure S3. Prey viability results used in plotting Figure 1b, showing expression of a functional *ompF* gene increases predation rates. Complementation of the Δ*ompF* knock-out led to similar predation rates as the wild-type *E. coli* BW25113 while over-expression of the *ompF* gene in the wild-type *E. coli* BW25113 background led to significantly better predation rates. In contrast, as shown in the bottom graph, loss of the *ompC* gene increased predation, while over-expression or complementation of this gene in *E. coli* suppressed predatory activities against this prey. The viabilities were measured after one hour of predation. (n = 3)
Figure S4. Representative microscopic images of the different isogenic mutants of *E. coli* BW25113 during predation. The predator (*B. bacteriovorus* 109J) expresses tdTomato, making it fluoresce red. Attachment was defined as a rod-shaped prey with the predator clearly attached to it, while bdelloplast formation was defined by prey cells that were spherical in shape with a fluorescent predator present within them. Scale bar – 5 μm.
Figure S5. Cell densities of the different *E. coli* BW25113 isogenic mutants. The results show all but *E. coli* BW25113 ΔompR have similar densities per OD. These values were used to calculate the PPR values. ** - $p < 0.01$ ($n = 3$)
Figure S6. Predation of a double (ΔompC ΔompF) mutant of *E. coli* BW25113. Much like *E. coli* JW091 (ΔompF), this double mutant is predated slowly. Complementation with a functional *ompF* gene significantly improves predation against this prey, illustrating the importance of OmpF in recognition of *E. coli*. (n = 3)
Figure S7. Impacts of NaCl during growth on the predation of the isogenic mutants of *E. coli* BW25113/pGen-luxCDABE. The data presented here is shown relative against the unpredated control (PPR = 0) at one hour. Much like *E. coli* JW0912 (ΔompF), *E. coli* JW3368 (ΔompR) is also predated slowly. In contrast, *E. coli* JW3367 (ΔenvZ) is predated better when the NaCl concentration in the LB medium during growth was higher. (n = 3)
Figure S8. Multiple sequence alignment of the different OmpF (and OmpK35) proteins.

The alignment was performed using the PROMALS3D server using the default parameters (17,18). The structure of OmpF of E. coli MH225 was retrieved from RCSB Protein Data Bank (https://www.rcsb.org) and used as the reference structure (Sequence “s001”). The first line in each block shows conservation indices for positions with a conservation index above 5. Each representative sequence has a magenta name and is colored according to PSIPRED (19).
(red: alpha-helix, blue: beta-strand). A representative sequence and the immediate sequences below it with black names form a closely related group. The last two lines show consensus amino acid sequence (Consensus_aa) and consensus predicted secondary structures (Consensus_ss). Consensus predicted secondary structure symbols: alpha-helix: h; beta-strand: e.

Conserved amino acids are in bold and uppercase letters; aliphatic (I, V, L): l; aromatic (Y, H, W, F): @; hydrophobic (W, F, Y, M, L, I, V, A, C, T, H): h; alcohol (S, T): o; polar residues (D, E, H, K, N, Q, R, S, T): p; tiny (A, G, C, S): t; small (A, G, C, S, V, N, D, T, P): s; bulky residues (E, F, I, K, L, M, Q, R, W, Y): b; positively charged (K, R, H): +; negatively charged (D, E): -; charged (D, E, K, R, H): c.
Figure S9. Expression of *E. coli* OmpF in *E. fergusonii* increases predation of this prey. As noted in Figure 2b, loss of the *ompF* homologue in *E. fergusonii* had no obvious impact on predation rates. However, expressing *ompF* from *E. coli* BW25113 within this prey led to better predation efficiencies, in both the wild-type and ΔompF genetic backgrounds. These results show OmpF in *E. coli* is being recognized by *B. bacteriovorus* 109J. (n = 3)
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