Supplemental Materials

Microcin PDI Inhibits Antibiotic-Resistant Strains of *Escherichia coli* and *Shigella* Through a Mechanism of Membrane Disruption and Protection by Homotrimer Self-Immunity

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Running head: Microcin PDI inhibits antibiotic-resistant bacteria

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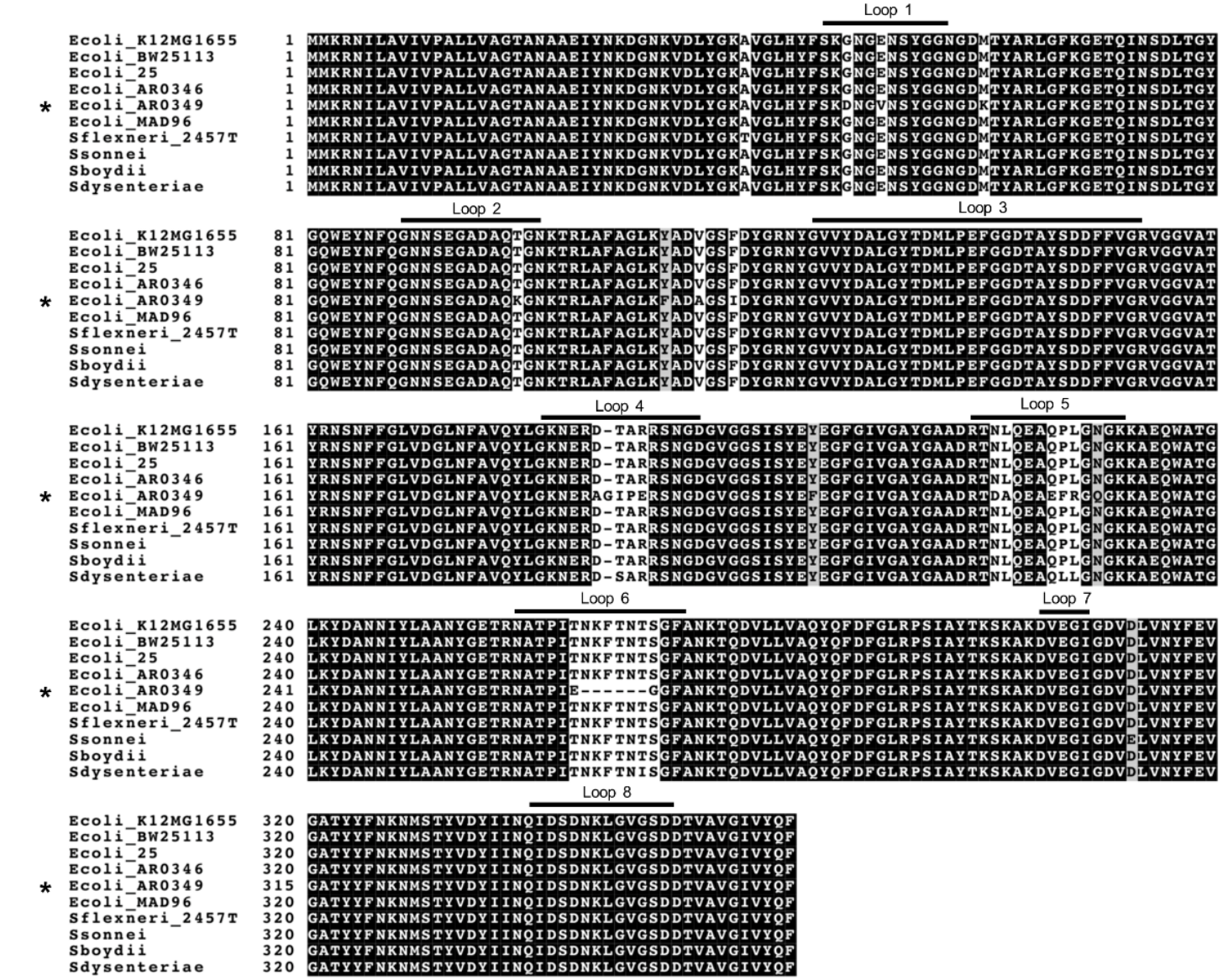
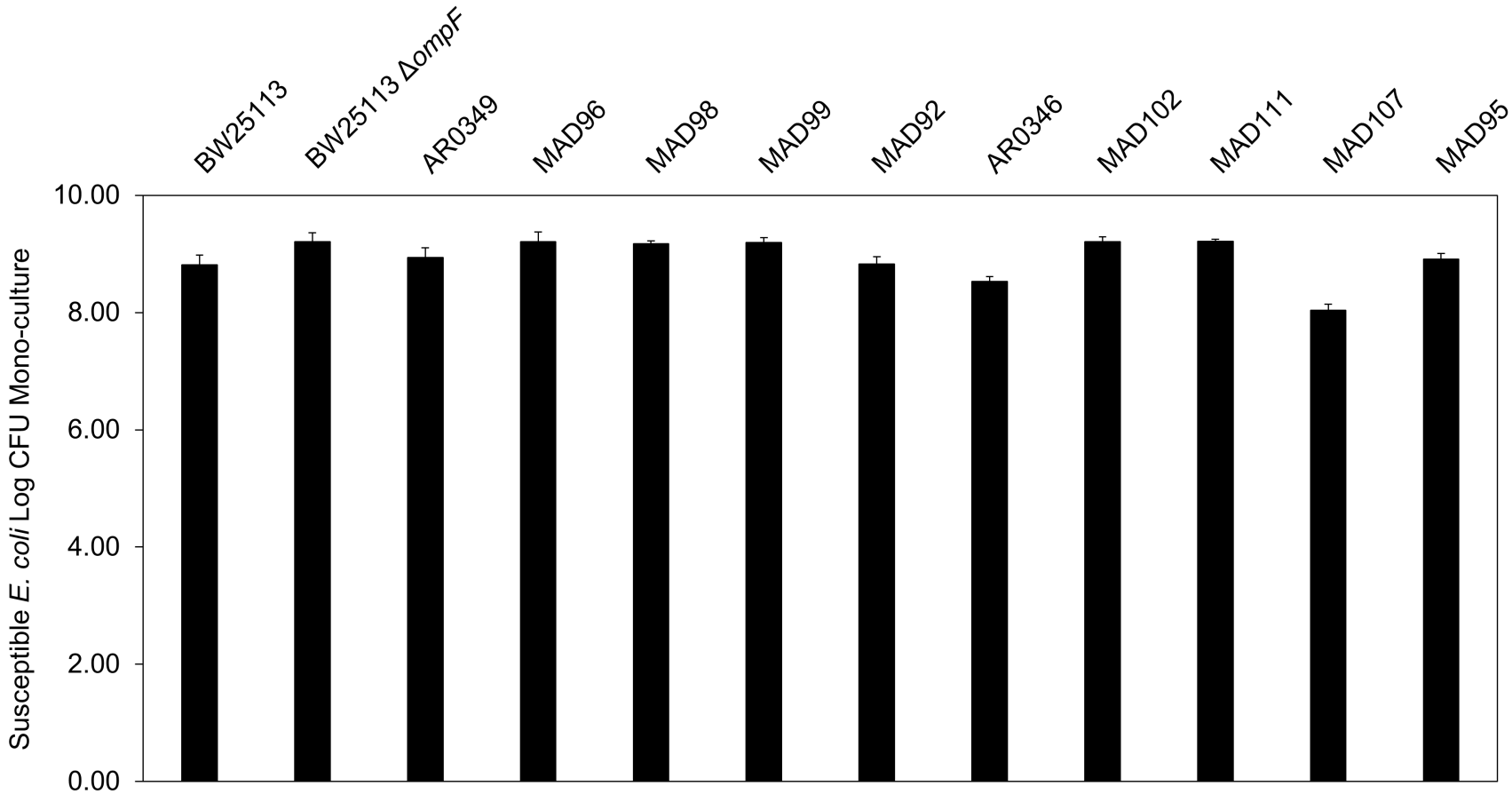
Supplemental Figure

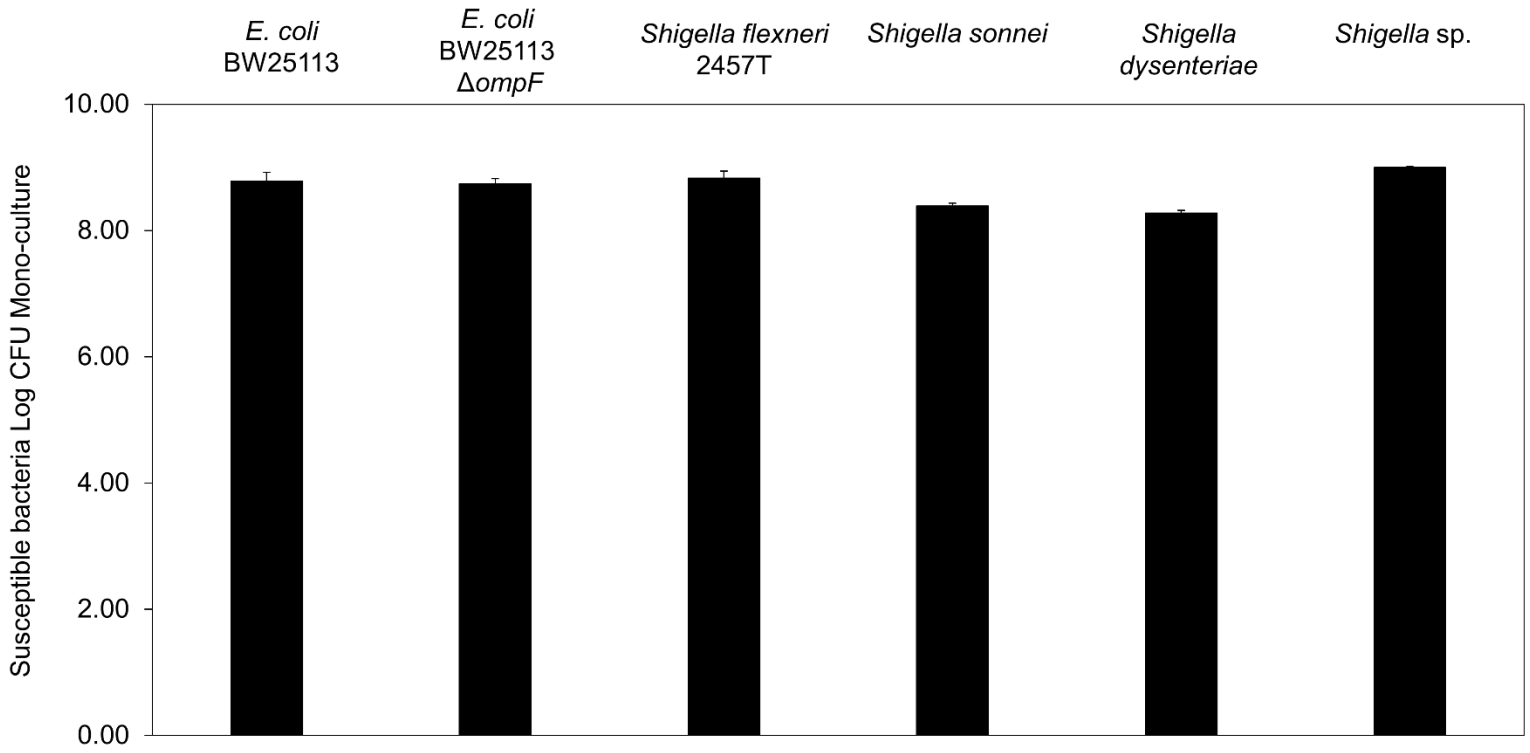


Supplemental Figure 1. Amino acid sequence alignment of OmpF. *E. coli*, *Shigella* strains, and MccPDI-resistant bacteria *E. coli* strains OmpF sequences alignment were generated using T-Coffee (1). Parenthetical numbers on the left indicate the amino acid position relative to the N terminus of each OmpF. The black shaded regions indicate completely conserved residues. Grey shaded regions are partially conserved residues with greater than 60% identity, while white regions are not conserved residues. Eight Extracellular loops (Loop 1 – 8) are marked based on *E. coli* OmpF crystal structure (2). *E. coli* K12 MG1655 (NP_415449), *E. coli* BW25113 (NZ_CP009273.1), *S. flexneri* 2a 2457T (AE014073.1) and *S. sonnei* 53G (HE616528.1), *S. boydii* CDC 3803-94 (CP001063.1), *S. dysenteriae* Sd197 (NC_007606.1), and *E. coli* AR0349 (MH665273) which encodes an OmpF differed significantly (*)

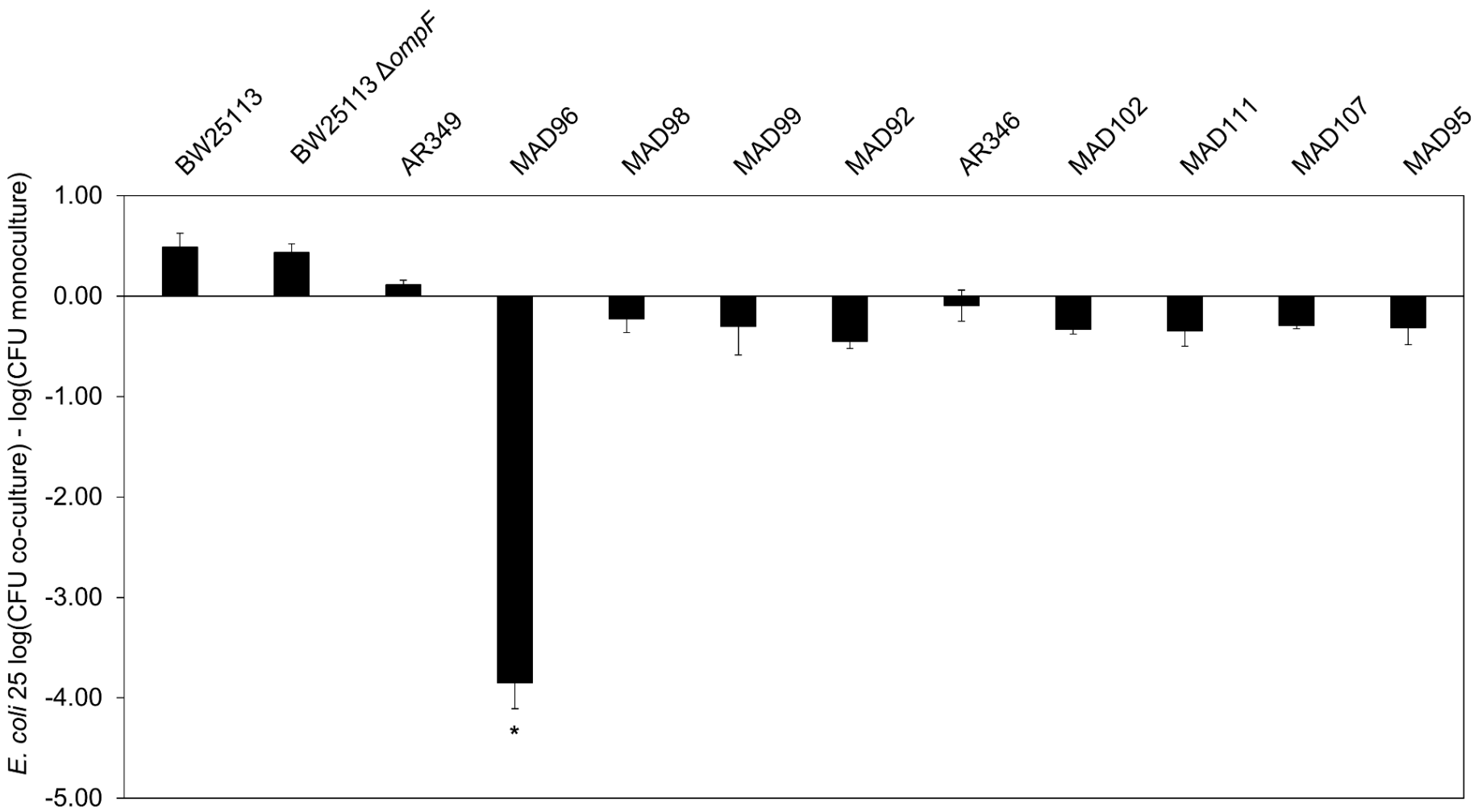
(A)


BW25113
BW25113 ΔompF
AR0349
MAD96
MAD98
MAD99
MAD92
AR0346
MAD102
MAD111
MAD107
MAD95
10.00
8.00
6.00
4.00
2.00
0.00
Susceptible E. coli Log CFU Mono-culture

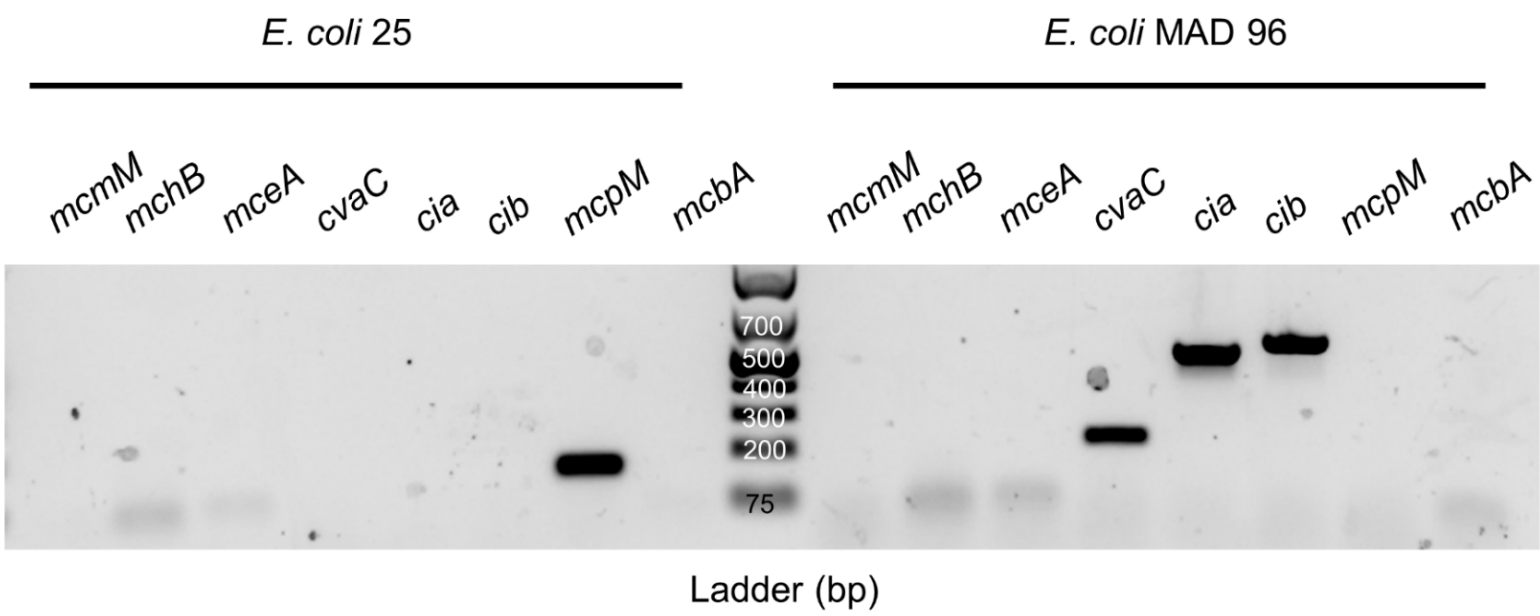
(B)



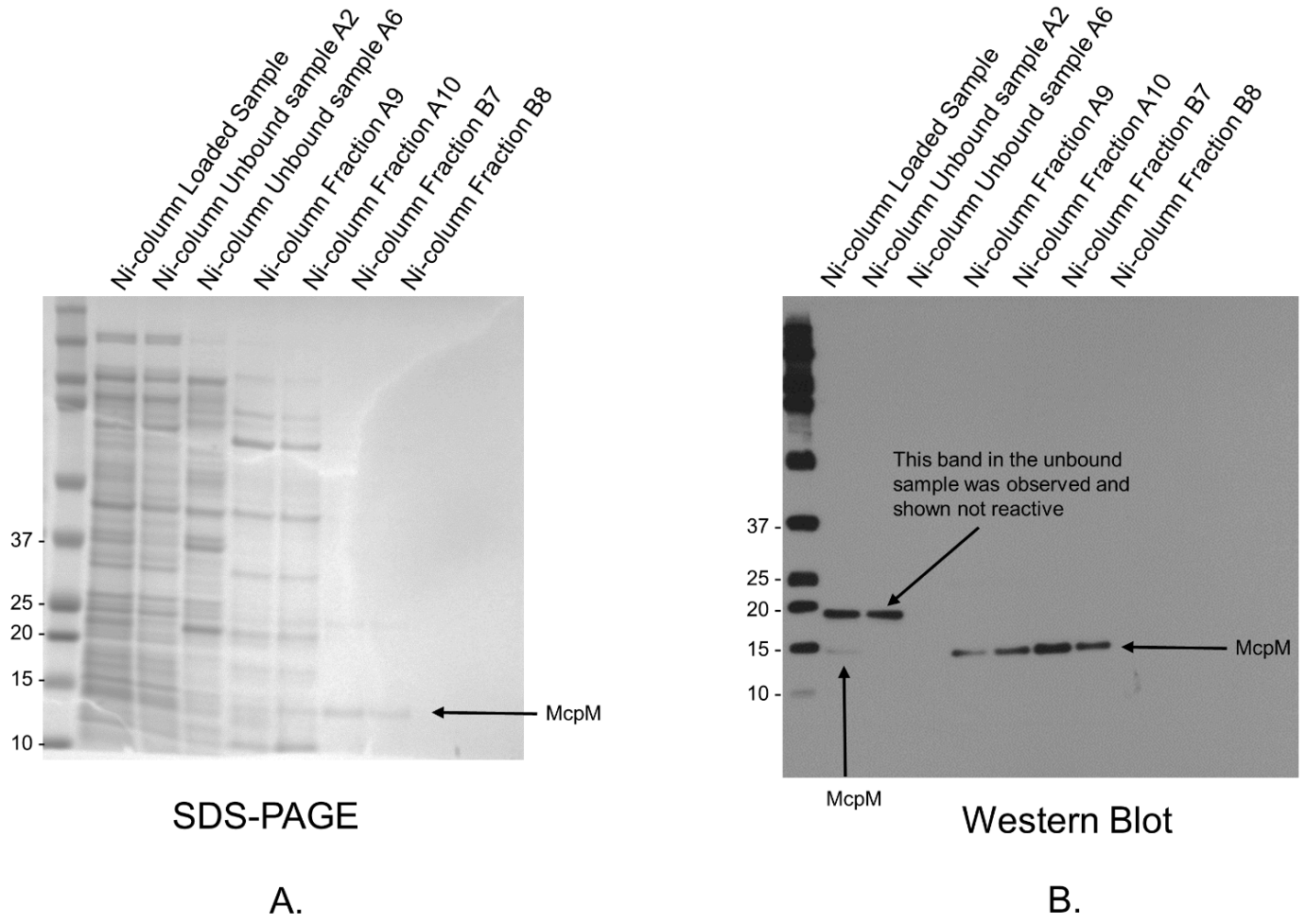
Supplemental Figure 2. UTI *E. coli* and *Shigella* strains monoculture without MccPDI exposure (A) Log_{10} transformed colony forming units (CFU/mL) for *E. coli* strains after 24 h culture in M9 media without exposure to an MccPDI-producing strain. (B) Log10 CFU/ml for *Shigella* strains (*flexerni* 24570, *flexerni* 2457T, *sonnei* WRAIR I virulent, *dysenteriae* Newcastle 1934, and Sp.) after 24 h culture in M9 media. Results are expressed as mean and standard error of the mean for three independent replicates.


BW25113
BW25113 ΔompF
AR349
MAD96
MAD98
MAD99
MAD92
AR346
MAD102
MAD111
MAD107
MAD95
1.00
0.00
-1.00
-2.00
-3.00
-4.00
-5.00
E. coli 25 log(CFU co-culture) - log(CFU monoculture)
*

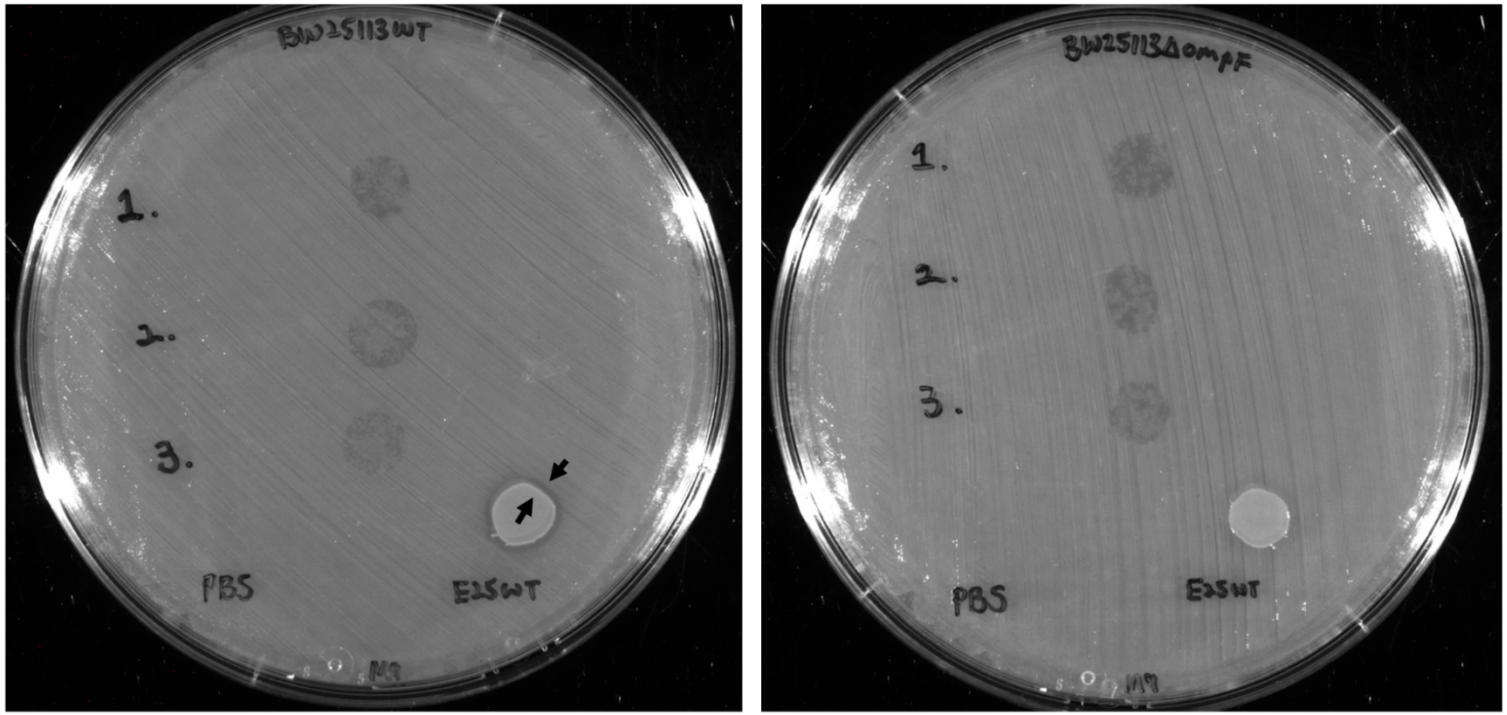
Supplemental Figure 3. *E. coli* 25 co-culture showed inhibition by MDR UPEC *E. coli* strain MAD 96. Co-culture competition of MccPDI producer (*E. coli* 25) against potential MccPDI susceptible *E. coli* strains. Results indicated all tested target *E. coli* strains against MccPDI did not inhibit MccPDI producer with the exception of *E. coli* strain MAD 96. Results are expressed as the difference of mean log CFU during co-culture and mono-culture of MccPDI producer strain *E. coli* 25 (n = 3 independent replicates; error bar = SEM).



Supplemental Figure 4. ***E. coli* strain MAD 96 PCR analysis tested positive for colicin Ia/Ib and microcin V.** *E. coli* 25 wild-type (*mcpM* positive) serves as a negative control for panels of known microcins and colisins genes. *E. coli* strain MAD 96 was tested positive for colisin Ia/Ib (*cia* and *cib*) and microcin V (*cvaC*). Genes tested consisted of: *mcmM* (microcin M), *mchB* (microcin H47), *mceA* (microcin E492), *cvaC* (microcin V), *cia* (colicin Ia), *cib* (colicin Ib), *mcpM* (microcin PDI), and *mcbA* (microcin B17).



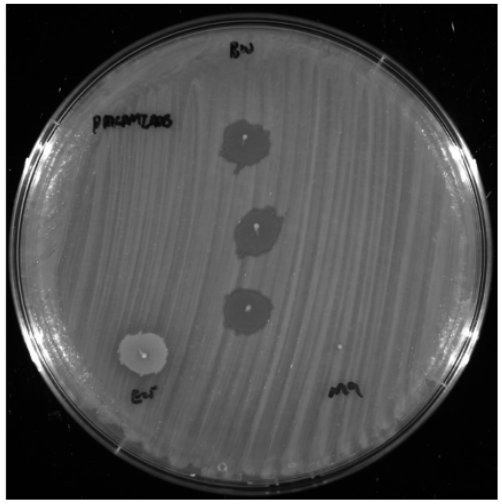
Supplemental Figure 5. HPLC Purified 6x His-tagged full length McpM. SDS-PAGE and Western blot were performed to visualize the purified McpM protein. (A) Sample fractions collected from the Ni-column (Unbound samples and fractions) were loaded onto a SDS-PAGE and stained with Coomassie Blue to visualize the presence of McpM 6x-His-tag band. (B). A Western blot was performed with the same samples as in (A) was detected with mouse anti-6xHis-tag and showed the presence of full length McpM peptide.



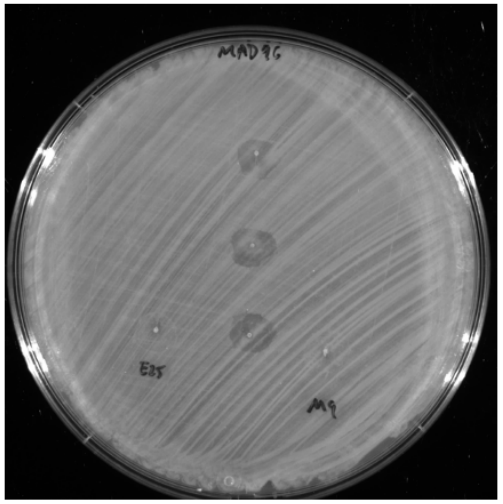
E. coli BW25113 wild-type

E. coli BW25113 Δ*ompF*

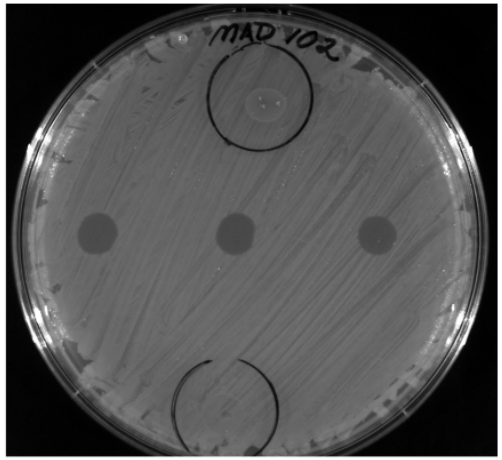
Supplemental Figure 6. Purified McpM demonstrated inhibition against susceptible *E. coli*. M9 Agar plate lawn with *E. coli* BW25113 (positive control) or BW25113 Δ*ompF* (negative control) was spotted with 5 µL of purified McpM with three technical replicates, *E. coli* 25 wild-type (MccPDI wild-type producer) positive control, and 1X sterile PBS (negative control). Plates were air dried and incubated overnight at 37°C. The result demonstrated inhibition against both strains in comparison to the positive control on susceptible strain (BW25113) indicated by zone of inhibition (black arrow).



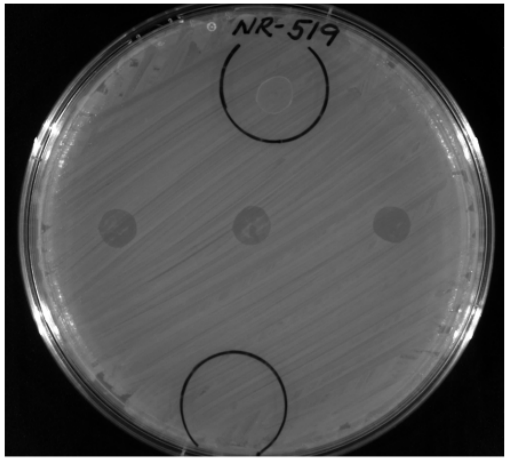
E. coli BW25113



E. coli MAD 96

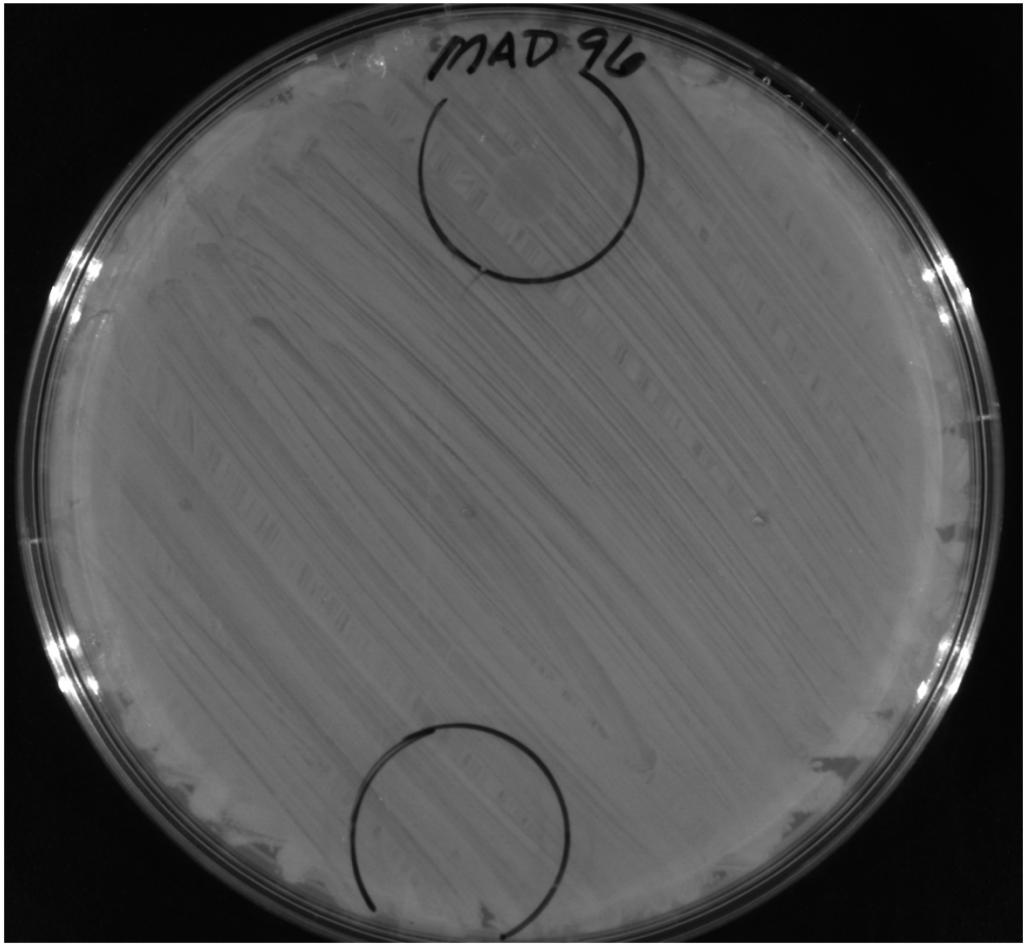


E. coli MAD 102

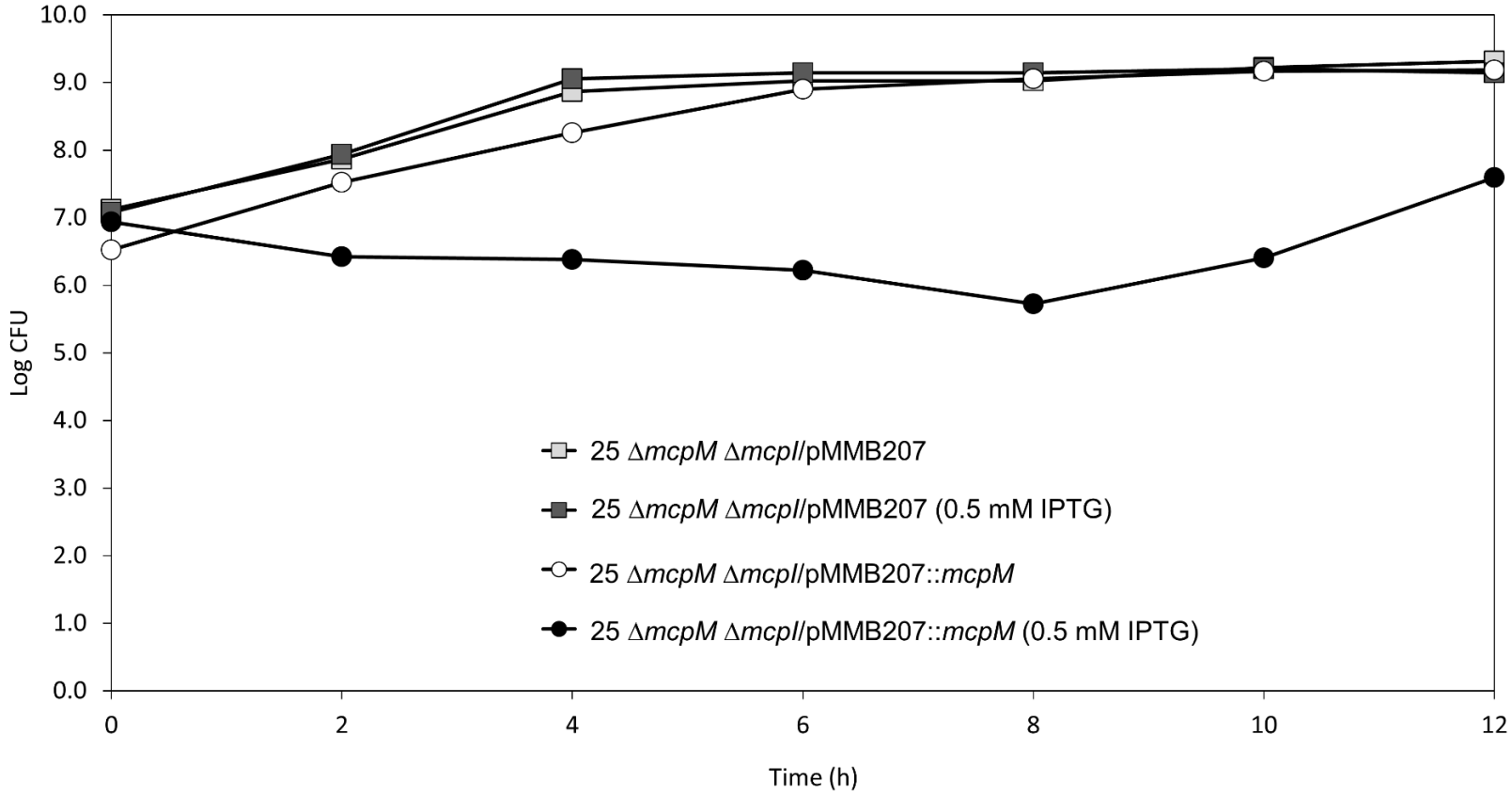


S. sonnei

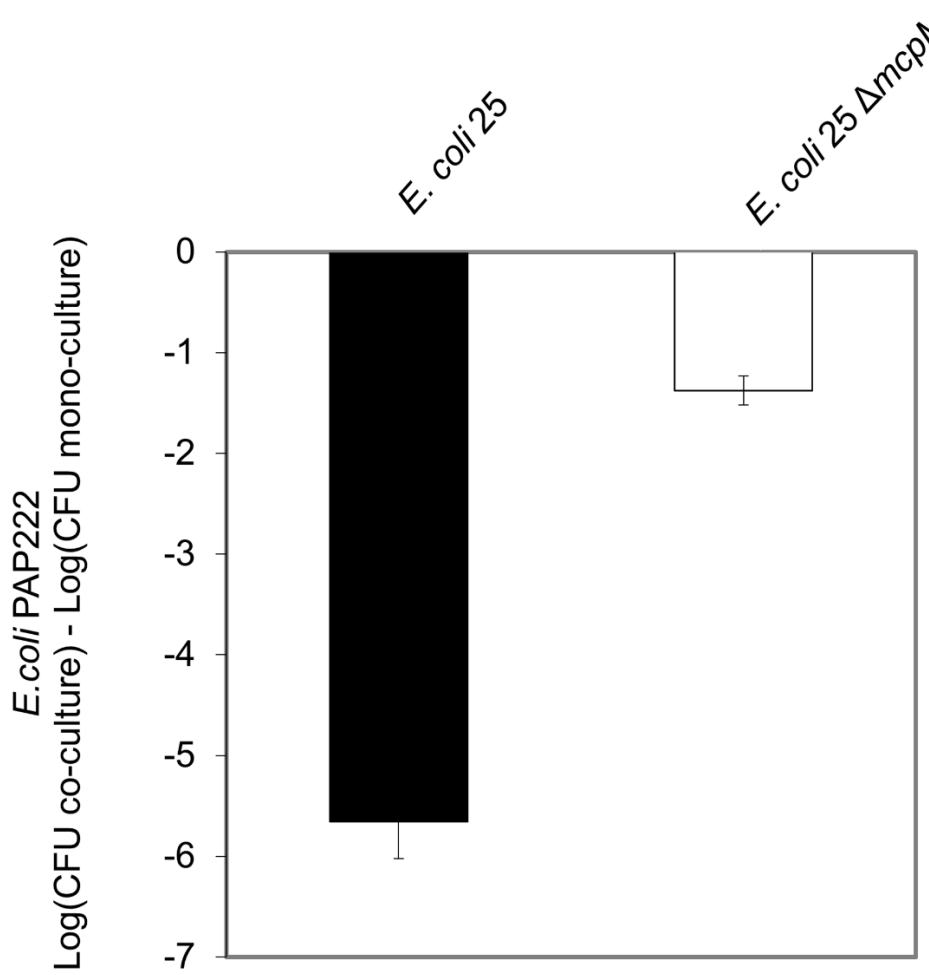
Supplemental Figure 7. McpM demonstrated inhibition against susceptible *E. coli* MAD96. LB Agar plate lawn with *E. coli* MAD96 (positive control) and was spotted with 5 µL of *E. coli* DH10B/pCR2.1::$p_{mic\text{-}210/0}$*mcpMIADB* with three technical replicates, *E. coli* 25 wild-type (MccPDI producer) negative control, and M9 media (negative control). Plate was air dried and incubated overnight at 37°C. The result demonstrated inhibition against *E. coli* strain BW25113 (positive control), MAD 96, MAD 102 and *Shigella sonnei* in comparison to the negative control strain 25 indicated by zone of inhibition.



Supplemental Figure 8. Purified McpM demonstrated inhibition against susceptible *E. coli* MAD96. LB Agar plate lawn with *E. coli* MAD96 (positive control) and was spotted with 5 µL of *E. coli* DH10B/pCR2.1::$p_{mic\text{-}210/0}$*mcpMIADB* with three technical replicates, *E. coli* 25 wild-type (MccPDI producer; upper circle) negative control, and M9 media (negative control; lower circle). Plate was air dried and incubated overnight at 37°C. The result demonstrated inhibition against *E. coli* strain 25.



Supplemental Figure 9. Log colony forming unit (Log CFU) of *E. coli* 25 Δ*mcpM* Δ*mcpI* complemented with pMMB207 or pMMB207::*mcpM* for 12 h. Log CFUs were measured for MccPDI producer (*E. coli* 25) with microcin (*mcpM*) and immunity (*mcpI*) gene knocked out complemented with empty vector or *mcpM* complement and induced with or without 0.5 mM IPTG in M9 defined media. Negative control are strains without *mcpM* gene complementation and without 0.5 mM IPTG induction. Bacterial culture taken every two hours for 12 h were plated onto LB agar plate with appropriate antibiotic and CFU numerated.



Supplemental Figure 10. Microcin V (MccV) producer (*E. coli* PAP222) was inhibited by MccPDI producer (*E. coli* 25) in co-culture competition. Compared to *E. coli* 25 Δ *mcpM* (white), *E. coli* 25 wild-type was able to inhibit *E. coli* PAP222 (MccV phenotype^{+}) strain (black) in M9 media for 24 h. Results are expressed as the difference of mean log CFU during co-culture and mono-culture of the target strain (n = 3 independent replicates; error bar = SEM).

Supplemental Figure 11. Predicted McpI structure. Based on the protein structure and function prediction (3), McpI is predicted to be a transmembrane protein that contains two transmembrane domains (4, 5).

REFERENCES

1. **Notredame C, Higgins DG, Heringa J.** 2000. T-Coffee: A novel method for fast and accurate multiple sequence alignment. J Mol Biol **302:**205-217.
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