# Supplementary Materials: pMPES: A Modular Peptide Expression System for the Delivery of Antimicrobial Peptides to the Site of Gastrointestinal Infections Using Probiotics

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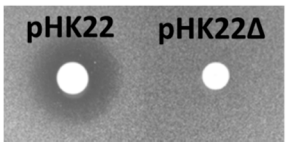


**Figure S1.** Abolishment of native Microcin V production by mutation of *cvaC*. Producer strain is *E. coli* MC1061 F′ and indicator strain is *E. coli* DH5α.

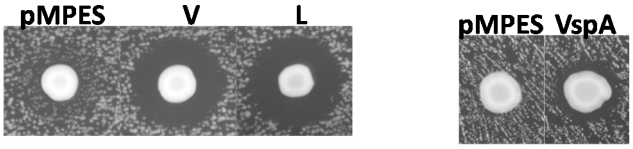


**Figure S2.** MccV, MccL, and EntA production from pMPES:V, L, and VspA in *E. coli* Nissle 1917. *E. coli* DH5α was used as the indicator strain for V and L and *E. faecium* 8E9 was used as the indicator strain for VspA. Note *E. coli* Nissle 1917 pMPES has native activity against *E. coli* DH5α but not *E. faecium* 8E9.

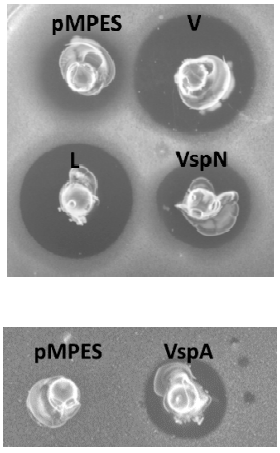


**Figure S3.** Alternative assay showing MccV, MccL, McnN and EntA production from pMPES:V, L, VspN, and VspA in *E. coli* Nissle 1917. *E. coli* DH5α was used as the indicator strain for V, L, and VspN and *E. faecium* 8E9 was used as the indicator strain for VspA. Note *E. coli* Nissle 1917 pMPES has native activity against *E. coli* DH5α but not *E. faecium* 8E9.

**Table S1.** Primers and DNA fragments used in this study.

| Primer | Sequence |
|---|---|
| SDML | CTGCGCGCATGGTCTTC |
| SDM R | GATAAAAAGGAGATCATTAAAGAACTCTGACTCTA |
| SDM F | TAGAGTCAGAGTTCTTTAATGATCT CCTTTTTATC |
| SDML R | TTGAGATCTGTTGAG AGGGGTTTT |
| SDM_seq_F | CAGCAGCTGCTCCAAT |
| SDM_seq_R | ATTGCATTAGCTATATATGATTGTGT |
| Proteon_Assembler_F | GACAGCTTATCATCGATACTAGATAGATGACCTCGGG |
| Proteon_Assembler_R | CTGGCATTATGGTGGAAAGCTTCAGTTTAATTAAG TGAGCTCACAGCTGTTTC |
| pHK22 HindIII Seq F | ATGTAGCACCTGAAGTCAGCCC |
| pHK22 HindIII Seq | GGTAATAGCGGTAAAGTGGCTAAACGG |
| MccV_SacI_F | TGCTAGAGCTCAAGTCAA GGCCGCATCG |
| MccV_PacI_R | CATAGTTAATTAATCTACAGTGAG CGGAAGGCC |
| AMP F | ATCCGGAGCTCTTAGCA |
| AMP R | TTGACTTAATTAATCGTAGGGGG |
| EntA_pHK22ΔP F | GAGAATTCAAGGAGGAAACAGCTGTGAGCTCTTAGCAGGAGGTGAACA TGAAGCATTTGAAGATCCTGAGC |
| EntA_pHK22ΔP R | GGACATGTCCGTAGCGGCCGCACTGACCTAGGTTAGAACTGACTTTTATC TCCATAGTCG |
| pMPES transition_F | GAGAATTCAAGGAGGAAACAGCTGTGAGCTCTTAGCAGGAGGTGAACA TGAGAACTCTGACTCTAAATGAATTAGATTCT |
| pMPES transition_R | ACATGTCCGTAGCGGCCGCACTGACCTAGGCTCATGGGCCCGTCAACTG TACGATCCTCTGAGCTTCG |
| MccV SeC F SpeI | CATAACTAGTATAGGAGGTTGTTTCGCCAGGAT |
| CvaB seq | ACAATCTGATCACAGGCGGT |
| PBS L_For | CTGATCATGCTCAAGGAGGAGTCAGATGAGAGAAATAACGTTAAATGA AATG |
| PBS L_Rev | CTGTACGATCCTCTGAGCTTCGACCTCATTTGCCACGGTTGAA |
| PBS VA_For | CTGATCATGCTCAAGGAGGAGTCAGATGAGAACTCTGACTCTAAATGAA TTAG |
| PBS VA_Rev | CTGTACGATCCTCTGAGCTTCGACCTTAGAACTGACTTTTATCTCCATAGT CG |
| R_For | AAGGAGGAAACAGCTGTGAGCTCCTGATCATGCTCAAGGAGGAGTCAG |
| O_Rev | GGGCCCTCTGTAACTACTGTGGGAGCTCCTGTACGATCCTCTGAGCTTCG ACC |
| O_For | CCACAGTAGTTACAGAGGGCCCCTGATCATGCTCAAGGAGGAGTCAG |
| YG_Rev | GCGGCCGCTATATGTTCTAGGCCTAGGGTCGCTATAGCTGCAGGGGCCC CTGTACGATCCTCTGAGCTTCGACC |

| DNA Fragments | Sequence [1] |
| --- | --- |
| ProTeOn+ | gacagcttatcatcgatactagatagatgacctggggagcccgcctaatgagcgggctttttgcgcgccactctatc<br>attgactctatcattgatagagtacttaacataagcacctgtaggatcgtacaggtttagcgaagaaaatggtttgtta<br>tagtcgaataaacctcgagttatctcgagtgagatattgttgacgcaccaaggaggaagcttctatgatgagccgtct<br>ggataaaagcaaagtgattaatagcgcactggaactgctgaatgaagttggtattgaaggtctgaccacccgtaaa<br>ctggcccagaaactgggtgttgaacagccgaccctgtattggcatgtgaaaaataaacgtgcactgctggatgcact<br>ggccgttgaaattctggctcgccatcatgattatagcctgcctgcagcaggcgaaagctggcagagctttctgcgta<br>ataatgccatgagctttcgtcgtgccctgctgcgttatcgtgatggtgcaaaagaacatctgggcacccgtccggatg<br>aaaaacagtatgataccgttgaaacccagctgcgttttatgaccgaaaatggttttagcctgcgtgatggtctgtatgc<br>aattagcgcagttagccattttaccctgggtgccgttctggaacagcaggaacataccgcagcactgaccgatcgtc<br>ctccggcaccggatgaaaatctgcctccgctgctgcgtgaagcactgatgattatggattctgatgatggtgaacag<br>gcatttctgcatggtctggaaagcctgattcgtggttttgaagttcagctgaccgcactgctgcagattgttggtggtg<br>gtggtgcacgtacccagtatagcgaaagcatgggtgcccgtacacagtattctgaatctatgggtgctcgcacccag<br>tattcagaaagtatgggtgcaagaacacagtatagcgagtctatgggagcgcgtactcagtatagtgaatcaatgg<br>gaggtggtatgccgagcctggttgataattaccgcaagattaatattgccaataataaaagcaacaacgatctgacc<br>aaacgtgaaaaagaatgtctggcctgggcatgtgaaggtaaaagcagctgggatattagcaaaattctgggttgta<br>gcgaacgtaccgtgacctttcatctgaccaatgcccagatgaaactgaataccaccaatcgttgtcagagcattagca<br>aagcaattctgaccggtgccattgattgtccgtatttcaaaaactgagaattcaaggaggaaacagctgtgagctca<br>cttaattaaactgaagctttccaccataatgccag |
| MccV | gtcaaggccgcatcgaggaggaaacagct**atgagaactctgactctaaatgaattagattctgtttctggtggtGC**<br>**TTCAGGGCGTGATATTGCGATGGCTATAGGAACACTATCCGGGCAAT**<br>**TTGTTGCAGGAGGAATTGGAGCAGCTGCTGGGGGTGTGGCTGGAGG**<br>**TGCAATATATGACTATGCATCCACTCACAAACCTAATCCTGCAATGTC**<br>**TCCATCCGGTTTAGGGGGAACAATTAAGCAAAAACCCGAAGGGATA**<br>**CCTTCAGAAGCATGGAACTATGCTGCGGGAAGATTGTGTAATTGGAG**<br>**TCCAAATAATCTTAGTGATGTTTGTTTATAA**gataccaggaggaaactgct*ATGGA*<br>*TAGAAAAAGAACAAAATTAGAGTTGTTATTTGCATTTATAATAAATGCCACC*<br>*GCAATATATATTGCATTAGCTATATATGATTGTGTTTTTAGAGGAAAGGACTT*<br>*TTTATCCATGCATACATTTTGCTTCTCTGCATTAATGTCTGCAATATGTTACTT*<br>*TGTTGGTGATAATTATTATTCAATATCCGATAAGATAAAAAGGAGATCATAT*<br>*GAGAACTCTGACTCTAAATGA*aggtccatggtacgtacccatagataggcgccgttatcgactgggc<br>ctcatgggccttccgctcactg |
| MccL | atccggagctcttagcaggaggtgaac**atgagagaaataacgttaaatgaaatgaataatgtctctggtgctGG**<br>**TGATGTCAATTGGGTTGATGTCGGGAAAACTGTAGCAACAAACGGTG**<br>**CAGGAGTGATTGGCGGTGCATTCGGAGCTGGTCTGTGCGGCCCTGTT**<br>**TGTGCTGGTGCCTTCGCTGTTGGATCTTCTGCCGCTGTTGCTGCTTTG**<br>**TATGATGCAGCAGGAAATTCCAACTCAGCGAAACAAAAACCAGAAG**<br>**GACTACCTCCAGAAGCATGGAACTACGCTGAAGGTAGAATGTGTAAT**<br>**TGGAGTCCAAATAATCTTAGTGATGTTTGTTTATAA**actggaggaggtgcac*AT*<br>*GAAAACTTGGCAGGTTTTCTTCATCATCCTTCCGATCTCCATCATTATCTCTC*<br>*TGATTGTTAAACAGCTTAACAGCTCCAACCTTGTACAGAGCGTTGTAAGCGG*<br>*CATTGCTATCGCACTCATGATCTCCATCTTTTTCAACCGTGGCAAATGA*ttgacg<br>ggcccatgagcctaggtcagtgcggccgctacggacatgtccgta*gctagcgaaaaaaaaacccgcccctgacagggc*<br>*gggttttttttttaatttaaccccc*tacgattaattaagtcaa |
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|---|---|
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| Lsp:EntA | atccggagctcttagcaggaggt**gaacatgagagaaataacgttaaatgaaatgaataatgtctctggtgct AC CACCCATAGCGGTAAGTATTACGGAAATGGAGTTTACTGTACCAAAA ATAAATGCACCGTTGATTGGGCTAAAGCGACAACTTGTATCGCTGGT ATGTCTATCGGCGGGTTCTTAGGGGGTGCCATTCCAGGCAAATGCTA A**tgctaaggaggtgcta*ATGAAGAAAAACGCCAAACAGATTGTTCACGAGTTATAT AACGACATTAGTATCAGCAAAGACCCGAAGTATTCGGACATCCTTGAGGTAC TGCAAAAAGTGTATCTGAAACTGGAAAAACAGAAATATGAGTTAGATCCAG GACCATTGATCAATCGTCTGGTAAACTACCTGTACTTCACCGCCTATACCAA CAAGATTCGTTTCACCGAATATCAAGAAGAGTTAATCCGCAATCTGTCTGAG ATTGGACGCACAGCAGGAATCAACGGACTGTACCGTGCCGACTATGGAGAT AAAAGTCAGTTCTAA* ttgacgggccc atgagcctagg tcagtgcggccgc tacggacatgt ccgta*gctagcgaaaaaaaaacccgcccctgacagggcgggttttttttttaatttaaccccccc*tacgattaattaagtcaa |
| Vsp:EntA (for pMPES) | gagaattcaaggaggaaacagctgtgagctcttagcaggaggtgaac**atgagaactctgactctaaatgaattag attctgtttctggtggtACCACTCATAGCGGTAAGTATTACGGAAATGGAGTTT ACTGTACCAAAAATAAATGCACCGTTGATTGGGCTAAAGCGACAACT TGTATCGCTGGTATGTCTATCGGCGGGTTCTTAGGGGGTGCCATTCC AGGCAAATGCTAA**tgctaaggaggtgcta*ATGAAGAAAAACGCCAAACAGATTGT TCACGAGTTATATAACGACATTAGTATCAGCAAAGACCCGAAGTATTCGGAC ATCCTTGAGGTACTGCAAAAAGTGTATCTGAAACTGGAAAAACAGAAATAT GAGTTAGATCCAGGACCATTGATCAATCGTCTGGTAAACTACCTGTACTTCA CCGCCTATACCAACAAGATTCGTTTCACCGAATATCAAGAAGAGTTAATCCG CAATCTGTCTGAGATTGGACGCACAGCAGGAATCAACGGACTGTACCGTGCC GACTATGGAGATAAAAGTCAGTTCTAA*ttgacgggcccatgagcctaggtcagtgcggccgct acggacatgtccgta*gctagcgaaaaaaaaacccgcccctgacagggcgggttttttttttaatttaaccccccc*tacgattaat taaactgaagctttccaccataatgcc |

| | |
|---|---|
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| Vsp:EntA (for pMPESb) | aaggaggaaacagctgtgagctcctgatcatgctcaaggaggagtcag**atgagaactctgactctaaatgaattag attctgtttctggtggt**ACCACTCATAGCGGTAAGTATTACGGAAATGGAGTTT **ACTGTACCAAAAATAAATGCACCGTTGATTGGGCTAAAGCGACAACT TGTATCGCTGGTATGTCTATCGGCGGGTTCTTAGGGGGTGCCATTCC AGGCAAATGCTAA**ggtcgaagctcagaggatcgtacaggagctcccacagtagttacagagggccc |
| Vsp:HirJM79 | aaggaggaaacagctgtgagctcctgatcatgctcaaggaggagtcag**atgagaactctgactctaaatgaattag attctgtttctggtggt**GCGACATACTATGGAAATGGATTGTATTGCAATAAGG **AGAAGTGTTGGGTCGATTGGAATCAAGCTAAAGGAGAGATCGGAAA GATAATAGTGAACGGTTGGGTGAATCATGGTCCTTGGGCTCCCAGAC GCTAA**ggtcgaagctcagaggatcgtacaggagctcccacagtagttacagagggccc |
| Vsp:EntP | ctgatcatgctcaaggaggagtcag**atgagaactctgactctaaatgaattagattctgtttctggtggt**GCAAC **TCGCTCATACGGGAATGGGGTGTATTGTAACAATTCTAAATGTTGGG TGAACTGGGGTGAAGCGAAAGAAAACATCGCTGGTATCGTCATCTCT GGCTGGGCCTCAGGACTTGCAGGAATGGGTCATTAA**ggtcgaagctcagagg atcgtacaggagctcccacagtagttacagagggccc |
| Vsp:EntB | aaggaggaaacagctgtgagctcctgatcatgctcaaggaggagtcag**atgagaactctgactctaaatgaattag attctgtttctggtggt**GAAAACGACCACAGAATGCCCAACGAGTTGAATCGCC **CTAACAATCTTAGCAAAGGGGGAGCCAAATGCGGCGCGGCGATTGC AGGTGGACTTTTCGGGATACCGAAAGGACCGCTGGCTTGGGCCGCT GGATTAGCGAATGTTTACTCAAAATGTAACTAA**ggtcgaagctcagaggatcgtac aggagctcccacagtagttacagagggccc |
| pMPES RBS | gagctcttagcaggaggtgaac |
| pMPESb RBS | gagctcctgatcatgctcaaggaggagtcag |

[1]: The following key is used to label components of AMP DNA fragments. Ribosomal Binding Sites: lowercased, underlined; Signal peptide: lowercased, bold; Mature peptide: uppercased, bold; Immunity Gene: uppercased, italicized; Terminator: lowercased, italicized, underlined.