# Supplemental information

# A colicin M-type bacteriocin from *Pseudomonas aeruginosa* targeting the HxuC heme receptor requires a novel immunity partner

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Table S1. Strains used in this study.

| Species | Strain | Characteristics/Source | Growth inhibition by PaeM4[a] | Presence *paeM4* locus | References |
|---|---|---|---|---|---|
| *Escherichia coli* | BL21(DE3) | Production of His-tagged bacteriocins | | | Novagen |
| | DH5α | Plasmid propagation | | | Invitrogen |
| *Pseudomonas aeruginosa* | CF_PA1 | CF patient | - | *pmiC* (orphan) | (1) |
| | CF_PA4 | CF patient | - | *pmiC* (orphan) | (1) |
| | CF_PA12 | CF patient | - | *pmiC* (orphan) | (1) |
| | CF_PA17 | CF patient | - | *pmiC* (orphan) | (1) |
| | CF_PA20 | CF patient | - | *pmiC* (orphan) | (1) |
| | CF_PA28 | CF patient | + | No | (1) |
| | CF_PA33 | CF patient | + | No | (1) |
| | CF_PA36 | CF patient | + | No | (1) |
| | CF_PA37 | CF patient | + | No | (1) |
| | CF_PA38 | CF patient | + | No | (1) |
| | CF_PA41 | CF patient | + | *pmiC* (orphan) | (1) |
| | CF_PA57 | CF patient | - | *pmiC* (orphan) | (1) |
| | CF_PA58 | CF patient | - | *pmiC* (orphan) | (1) |
| | CF_PA59 | CF patient | - | *pmiC* (orphan) | (1) |
| | CF_PA62 | CF patient | + | No | (1) |
| | CF_PA79 | CF patient | + | No | (1) |
| | CF_PA86 | CF patient | + | No | (1) |
| | CF_PA87 | CF patient | - | *pmiC* (orphan) | (1) |
| | CF_PA94 | CF patient | + | No | (1) |
| | CF_PA103 | CF patient | + | No | (1) |
| | CF_PA116 | CF patient | + | No | (1) |
| | CF_PA117 | CF patient | + | No | (1) |
| | Pr335 | Hospital environment | + | No | (2) |
| | W5 Aug28 | River water | + | No | (3) |
| | LMG 2107 | Shallow well water | + | No | (2) |
| | W15 Apr4 | River water | + | No | (4) |
| | Bo559 | Burn wound | + | No | (2) |
| | Jp97 | Sea water | T | No | (5) |
| | Jp100 | Sea water | T | No | (4) |
| | TA08 | Sputum | + | No | (5) |
| | Is580 | Burn wound | + | No | (2) |
| | A19 | Wound | + | No | (5) |
| | W15 Dec4 | River water | + | No | (3) |
| | W15 Oct31 | River water | + | No | (4) |
| | Br776 | Throat | - | *pmiC* (orphan) | (5) |
| | Br670 | Sputum | + | No | (2) |
| | Br906 | Nose | + | No | (2) |

| | | | | | |
|---|---|---|---|---|---|
| | Li004 | CF patient | - | *pmiC* (orphan) | (2) |
| | Is579 | Burn wound | - | *pmiC* (orphan) | (2) |
| | Br908 | Throat | + | No | (2) |
| | CPHL 6750 | Urine | + | No | (5) |
| | CPHL 2000 | Wound | + | *pmiC* (orphan) | (5) |
| | Us448 | Urine | + | No | (2) |
| | TuD47 | Ascite | - | *pmiC* (orphan) | (2) |
| | Br262 | Tap water room burn wound center | + | No | (5) |
| | Bu002 | Wound | - | *pmiC* (orphan) | (5) |
| | Pr317 | Burn | - | *pmiC* (orphan) | (5) |
| | Pr334 | Hospital environment | - | *pmiC* (orphan) | (5) |
| | W15 Dec14 | River water | - | *pmiC* (orphan) | (3) |
| | Is582 | Burn wound | - | *pmiC* (orphan) | (5) |
| | Is573 | Burn wound | - | *pmiC* (orphan) | (2) |
| | So092 | Burn wound | - | *pmiC* (orphan) | (2) |
| | Aa246 | Burn wound | + | No | (2) |
| | Br678 | Burn wound | - | *pmiC* (orphan) | (5) |
| | So123 | Hospital environment | + | No | (5) |
| | Br817 | Wound | - | *pmiC* (orphan) | (5) |
| | Bo562 | Blood | - | *pmiC* (orphan) | (5) |
| | So098 | Wound | - | *pmiC* (orphan) | (2) |
| | Br993 | Sputum | + | No | (2) |
| | Br229 | Hospital | + | No | (2) |
| | Br680 | Burn wound | + | No | (2) |
| | PA7 | Wound | + | No | (6) |
| | Us451 | Burn wound | - | *paeM4-pmiC* | (5) |
| | ATCC 27853 | Blood | + | No | (2) |
| | Bu007 | Burn wound | - | *pmiC* (orphan) | (2) |
| | PAO1 | Wound | + | No | (7) |
| | PA229 | Clinical isolate | - | *pmiC* (orphan) | (8) |
| | PA62 | Clinical isolate | - | *pmiC* (orphan) | (9) |
| | PA63 | Clinical isolate | - | *pmiC* (orphan) | (10) |
| | PABL056 | Blood | - | *pmiC* (orphan) | (11) |
| | HB15 | Sputum | - | *pmiC* (orphan) | (12) |
| | SCV20265 | CF patient | - | *paeM4-pmiC* | (13) |
| | LMG 1242 | Source unknown | - | *pmiC* (orphan) | (14) |
| | LMG 1272 | Cultivated mushroom | + | No | (10) |
| | PA45 | Blood | + | No | (15) |
| Transposon mutants *P. aeruginosa* PAO1 | | | | | |
| | PA0151 | PAO1 mutant hit in PA0151 | + | No | (16) |
| | PA0192 | PAO1 mutant hit in PA0192 | + | No | (16) |
| | PA0434 | PAO1 mutant hit in PA0434 | + | No | (16) |
| | PA0470 | PAO1 mutant hit in PA0470 (*fiuA*) | + | No | (16) |

| | | | | | |
|---|---|---|---|---|---|
| | PA0674 | PAO1 mutant hit in PA0674 (*pigC*) | + | No | (16) |
| | PA0781 | PAO1 mutant hit in PA0781 | + | No | (16) |
| | PA0931 | PAO1 mutant hit in PA0931 (*pirA*) | + | No | (16) |
| | PA1271 | PAO1 mutant hit in PA1271 | + | No | (16) |
| | PA1302 | PAO1 mutant hit in PA1302 (*hxuC*) | - | No | (16) |
| | PA1322 | PAO1 mutant hit in PA1322 (*pfuA*) | + | No | (16) |
| | PA1365 | PAO1 mutant hit in PA1365 | + | No | (16) |
| | PA1613 | PAO1 mutant hit in PA1613 | + | No | (16) |
| | PA1910 | PAO1 mutant hit in PA1910 (*femA*) | + | No | (16) |
| | PA1922 | PAO1 mutant hit in PA1922 | + | No | (16) |
| | PA2057 | PAO1 mutant hit in PA2057 | + | No | (16) |
| | PA2070 | PAO1 mutant hit in PA2070 | + | No | (16) |
| | PA2089 | PAO1 mutant hit in PA2089 | + | No | (16) |
| | PA2289 | PAO1 mutant hit in PA2289 | + | No | (16) |
| | PA2335 | PAO1 mutant hit in PA2335 (*optO*) | + | No | (16) |
| | PA2398 | PAO1 mutant hit in PA2398 (*fpvA*) | + | No | (16) |
| | PA2466 | PAO1 mutant hit in PA2466 (*optS*) | + | No | (16) |
| | PA2590 | PAO1 mutant hit in PA2590 | + | No | (16) |
| | PA2688 | PAO1 mutant hit in PA2688 (*pfeA*) | + | No | (16) |
| | PA2911 | PAO1 mutant hit in PA2911 | + | No | (16) |
| | PA3268 | PAO1 mutant hit in PA3268 | + | No | (16) |
| | PA3408 | PAO1 mutant hit in PA3408 (*hasR*) | + | No | (16) |
| | PA3790 | PAO1 mutant hit in PA3790 (*oprC*) | + | No | (16) |
| | PA3901 | PAO1 mutant hit in PA3901 (*fecA*) | + | No | (16) |
| | PA4156 | PAO1 mutant hit in PA4156 | + | No | (16) |
| | PA4168 | PAO1 mutant hit in PA4168 (*fpvB*) | + | No | (16) |
| | PA4221 | PAO1 mutant hit in PA4221 (*fptA*) | + | No | (16) |
| | PA4514 | PAO1 mutant hit in PA4514 (*piuA*) | + | No | (16) |
| | PA4675 | PAO1 mutant hit in PA4675 | + | No | (16) |
| | PA4710 | PAO1 mutant hit in PA4710 (*phuR*) | + | No | (16) |
| | PA4837 | PAO1 mutant hit in PA4837 | + | No | (16) |
| | PA4897 | PAO1 mutant hit in PA4897 (*optI*) | + | No | (16) |

[a] Growth inhibition by PaeM4, +; no growth inhibition by PaeM4, -. A non-transparent halo indicative of reduced cell density compared with the cell lawn is marked with T (turbid halo).

Table S2. List of plasmids used in this study.

| Plasmid | Characteristics | Reference |
|---|---|---|
| pET28a(+) | pBR322 origin, His-tag/thrombin/T7 tag; $Km^R$ | Novagen |
| pJB3Tc20 | Broad-host-range cloning vector, IncP; $Ap^R$, $Tc^R$ | (17) |
| pMMB67EH | Broad-host-range cloning vector, IncQ; $Gm^R$ | (18) |
| | | |
| pCMPG6250 | pET28a(+) with 866-bp PCR-amplified fragment containing *paeM* (locus tag ERS445055_00256) from *P. aeruginosa* NCTC 10332 cloned in NcoI/XhoI | (19) |
| pCMPG6252 | pJB3Tc20 with 467-bp PCR-amplified containing *pmiA* (no annotation of immunity gene downstream of *paeM*) from *P. aeruginosa* NCTC 10332 cloned in PstI/EcoRI | (19) |
| pCMPG6283 | pET28a(+) with 1025-bp PCR-amplified fragment containing *paeM4* (locus tag Q057_04089) from *P. aeruginosa* BL03 cloned in NcoI/XhoI | This study |
| pCMPG6287 | pJB3Tc20 with 739-bp PCR-amplified containing *pmiC* (locus tag Q057_04090) from *P. aeruginosa* BL03 cloned in PstI/EcoRI | This study |
| pCMPG6293 | pMMB67EH with 2727-bp PCR-amplified fragment containing *hxuC* (locus tag PA1302) from *P. aeruginosa* PAO1 cloned in EcoRI/HindIII | This study |

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