### SUPPLEMENTAL DATA

| Bacterial Strains | Locus Tag | Description / genotype |
|-------------------|-----------|------------------------|
| **pqsA**          | PA0996    | pqsA-H05::lacZbp02q1   |
| **pqsH**          | PA2587    | pqsH-F01::lacZbp03q3   |
| **pqsL**          | PA4190    | pqsL-H11::lacZbp02q4   |
| **lasI**          |           | Deletion mutant in PAO1 background |
| **rhlI**          |           | Deletion mutant in PAO1 background |
| **lasI rhlI**     |           | Deletion mutant in PAO1 background |
| **vgrG**          | PA0091    | vgrG1-D02::phoAwp01q2  |
| **Hcp**           | PA0263    | HcpC-E04::phoAwp04q3   |
| **cplV**          | PA0090    | cplV1-F02::phoAwp02q4  |
| **cdiA**          | PA2462    | PA2462-G12::lacZwp02q4 |
| **cdiB**          | PA2463    | PA2463-A05::phoAwp03q4 |
| **pcrV**          | PA1706    | pcrV-F02::lacZbp02q3   |
| **pcrD**          | PA1703    | pcrD-A09::phoAwp01q1   |
| **exoT**          | PA0044    | exoT-C09::lacZwp03     |
| **fliC**          | PA1092    | fliC-G10::phoAwp05q2   |
| **pilA**          | PA4525    | pilA-E01::lacZwp08q4   |
| **fliC pilA**     |           | Double mutant in the MPAO1 background (Parsek Lab) |
| **pchE**          | PA4226    | pchE-E06::phoAwp07q4   |
| **pvdD**          | PA2399    | pvdD-E07::lacZbp02q4   |
| **xcpR**          | PA3013    | xcpR-D09::phoAwp03q3   |
| **xcpQ**          | PA3105    | xcpQ-E03::phoAbp02q4   |

Table S1. *P. aeruginosa* mutant strain
**A.**

MaS Log10 CFU/mL

- **MaS only**
- **MaS in co-culture with PAO1**

**B.**

MaR Log10 CFU/mL

- **MaR only in 7H9**
- **MaR + PAO1 in 7H9**
- **MaR + PAO1 in 7H9 + Tw**

**C.**

PAO1 Log10 CFU/mL

- **PAO1 only in 7H9**
- **PAO1 only in 7H9 + Tw**
- **MaR + PAO1 in 7H9**
- **MaR + PAO1 in 7H9 + Tw**
Figure S1. (A) $\log_{10} \text{CFU/mL}$ of MaS grown alone versus in co-culture with PAO1 for 72 hrs. There was no statistical significance between the two conditions. (B) $\log_{10} \text{CFU/mL}$ of MaR grown alone versus in co-culture with PAO1 for 72 hrs. in 7H9 vs 7H9 + Tween. There was no statistical significance between the two conditions. (C) $\log_{10} \text{CFU/mL}$ of PAO1 grown alone versus in co-culture with MaR for 72 hrs. in 7H9 vs 7H9 + Tween. There was no statistical significance between the two conditions. (D) $\log_{10} \text{CFU/mL}$ of MaR at OD$_{600}$ of 0.150 grown alone versus in co-culture with PAO1 at different starting OD$_{600}$ values of 0.05, 0.150 and 0.5 for 72 hrs. There was no statistical significance between the two conditions. (E) $\log_{10} \text{CFU/mL}$ of PAO1 at different starting OD$_{600}$ values of 0.05, 0.150 and 0.5 grown alone versus in co-culture with MaR at OD$_{600}$ of 0.150 for 72 hrs. There was no statistical significance between the two conditions. (F) $\log_{10} \text{CFU/mL}$ of MaR grown alone versus in co-culture with PAO1 for 72 hrs. with 1mL vs. 2mL media replenished every 24hrs. There was no statistical significance between the two conditions. (G) $\log_{10} \text{CFU/mL}$ of PAO1 grown alone versus in co-culture with MaR for 72 hrs. with 1mL vs. 2mL media replenished every 24hrs. There was no statistical significance between the two conditions. N=2 for all data shown.
A. MaR alone MaR + heat killed PAO1

B. MaR/MaS Log10 reduction

C. MaS alone (1:1) MaS + PAO1 (1:1) MaS alone (100:1) MaS + PAO1 (100:1)

D. MaR alone (1:1) MaR + PAO1 (1:1) MaR alone (100:1) MaR + PAO1 (100:1)

E. MaS Log10 Reduction

- MaS
- MaS + PA01
**Figure S2.** (A) Log$_{10}$ CFU graph of heat-killed *P. aeruginosa* biofilm with MaR and MaS. Two filters were placed on each plate with two biological replicates. There was no statistical significance between the two conditions. (B) Log$_{10}$ reduction graph of MaR, MaS with *P. aeruginosa* biofilm in six-well plate in the absence of a membrane filter. There were two technical and biological replicates for each condition (N=2). Unpaired t-test **** P<0.0001. (C) Log$_{10}$ reduction graph of MaS with PAO1 biofilm at MOI of 1:1 and 100:1 respectively. Two filters were placed on each plate with two biological replicates (N=2). Unpaired t-test **** P<0.0001. (D) Log$_{10}$ reduction graph of MaR with PAO1 biofilm at MOI of 1:1 and 100:1 respectively. Two filters were placed on each plate with two biological replicates (N=2). Unpaired t-test **** P<0.0001. (E) Log$_{10}$ reduction CFU graph of kinetic biofilm over 6 days comparing *P. aeruginosa* growth in single-species biofilm to dual-species biofilm with MaS. Two filters were placed on each plate with three biological replicates. Unpaired T-test, *** P <0.001, **** P<0.0001 (N=2). (F) Log$_{10}$ reduction graph of MaR grown in a single species biofilm compared to growth in the presence of diluted PAO1 supernatant (N=2). Unpaired t-test ** P=00034. (G) Log$_{10}$ reduction graph MaR grown in a single species biofilm compared to growth in the presence of diluted PAO1 and MaR supernatant (N=2). There is no statistical significance. (H) Log$_{10}$ reduction graph of MaS grown in a single species biofilm compared to growth in the presence of diluted PAO1 supernatant (N=2). There was no statistical significance. (I) Log$_{10}$ reduction graph MaS grown in a single species biofilm compared to growth in the presence of diluted PAO1 and MaS supernatant (N=2). There was no statistical significance. (J) Antagonism of MaR was observed by either scraping an agar plate to dilute to the desired OD or preparing an overnight culture diluted to the desired OD. Two filters were placed on each plate with two biological replicates (N=2). Unpaired t-test **** P<0.0001.
| Gene   | MaS only | PAO1 | pqsA | pqsH | pqsL | lasI | rhlI | lasIrhlI | XcpR | XcpQ |
|--------|----------|------|------|------|------|------|------|----------|------|------|
|        | +        | +    | +    | +    | +    | +    | +    | +        | +    | +    |

![Graph showing MaS Log 10 Reduction](image-url)
**B.**

**MaS only**

| Gene    | Presence |
|---------|----------|
| PAO1    |          |
| vgrG    |          |
| Hcp     |          |
| cplV    |          |
| pcrV    |          |
| pcrD    |          |
| ExoT    |          |
| cdlA    |          |
| cdlB    |          |

**MaS Log 10 Reduction**

![Bar chart showing log reduction of MaS with various gene combinations.]

**MaS only**

| Gene    | Presence |
|---------|----------|
| PAO1    |          |
| filC    |          |
| pilA    |          |
| pilCpilA|          |
| pvdD    |          |
| pchE    |          |

**MaS Log 10 Reduction**

![Bar chart showing log reduction of MaS with various gene combinations.]

B ********

C ********
Figure S3. (A) Log_{10} reduction graph of MaS single-species biofilm growth compared to dual-species biofilm with *P. aeruginosa* QS (*lasI, rhlI, and lasIrhlI*), PQS biosynthesis mutants (*pqxA, pqxL, and pqxH*) and T2SS mutants (*xcpR and xcpQ*). (N=3). Unpaired t-test **** P<0.0001 (Unpaired t-test comparing PAO1 to *pqxA, pqxH* and *lasI* mutants* P=0.0112, ** P=0.0083, and *P=0.0105 respectively). (B) Log_{10} reduction graph of MaS single-species biofilm growth compared to dual-species biofilm with *P. aeruginosa* T6SS mutants (*vgrG, hcp, and cplV*), T3SS (*pcrV, pcrD, and exoT*) and CDI mutants (*ΔcdiAPA0041* and *ΔcdiBPA2463*). (N=2). Unpaired t-test **** P<0.0001. (C) Log_{10} reduction of MaS single-species biofilm growth compared to dual-species biofilm with *P. aeruginosa* motility mutants (*fliC, pilA*, and *fliCpilA*) and iron sequestering mutants (*pvdD and pchE*) (N=2). Unpaired t-test **** P<0.0001. All experiments evaluated two technical replicates and two biological replicates except for with three biological replicates.
|        | PAO1 | MaR  | MaS  |
|--------|------|------|------|
| **PIA** | ![Image](image1.png) | ![Image](image2.png) | ![Image](image3.png) |
| **7H10** | ![Image](image4.png) | ![Image](image5.png) | ![Image](image6.png) |
| **7H10 + Kanamycin** | ![Image](image7.png) | ![Image](image8.png) | ![Image](image9.png) |

**A.**

|        | 0h   | 24h  | 48h  | 72h  |
|--------|------|------|------|------|
| ![Image](image10.png) | ![Image](image11.png) | ![Image](image12.png) | ![Image](image13.png) |
Figure S4. (A) MaS, MaR and PAO1 streaked on PIA, 7H10 plate and 7H10 + Kan plates. PIA plates incubated for 1 day since CFUs are counted on PIA plates are after 1 day of incubation and 7H10 and 7H10 + Kan plates incubated for 4 days. (D) Image showing the growth of MaR in liquid culture over 72hrs. Images were taken every 24hrs.