Supplemental Material

The Impact of a Transposon Insertion in \textit{phzF2} on the Specialized Metabolite Production and Interkingdom Interactions of \textit{Pseudomonas aeruginosa}

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Running Title: Impact of \textit{phzF2} on Specialized Metabolite Production

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Figure S1. *P. aeruginosa* has two *phzF* genes (*phzF1* and *phzF2*) with 100% identity. To determine whether the transposon insertion was in *phzF1* or *phzF2*, primers were designed to specifically amplify each *phzF* gene. M: marker, 1: PAO1 with amplification of the *phzF1* gene (expected product 3.7 kb), 2: the *phzF2* mutant with amplification of the *phzF1* gene (expected product 3.7 kb), 3: PAO1 with amplification of the *phzF2* gene (expected product 2.7 kb), 4: the *phzF2* mutant with amplification of the *phzF2* gene (expected product 2.7 kb).
**Figure S2.** Duplicate MALDI FT-ICR imaging data of the interactions between *P. aeruginosa* PAO1 or the *phzF2* mutant and *A. fumigatus* Af293. The detected mass range was 175 to 375. Spatial resolution is 200 μm. Region of interest corresponds to the area measured. High resolution MALDI IMS is less sensitive than traditional IMS. 5-MPCA was not detected in the *P. aeruginosa – A. fumigatus* interaction because it

<table>
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<th>m/z</th>
<th>Description</th>
<th>PAO1-PA293</th>
<th>PhzF2-PA293</th>
<th>PAO1</th>
<th>PhzF2</th>
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<tr>
<td>899</td>
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<tr>
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<td>TAFC [Fe^{3+}]</td>
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<tr>
<td>1334</td>
<td>pyoverdine</td>
<td></td>
<td></td>
<td>NA*</td>
<td>NA*</td>
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<tr>
<td>1390</td>
<td>pyoverdine [Fe^{3+}]</td>
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was produced at levels below the limit of detection for this mass spectrometer. NA* denotes not analyzed.

Table S1. Metabolite Production by PAO1 and the *phzF2* Mutant in an Interaction with Af293

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>MPAO1-Af923 Peak Area</th>
<th>PhzF2-Af293 Peak Area</th>
<th>Fold Change</th>
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<td>1-HP</td>
<td>1348479</td>
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<td>+6.5</td>
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<td>PCA</td>
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<td>112756</td>
<td>-15.4</td>
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<td>5-MPCA*</td>
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<td>+5.6</td>
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<tr>
<td>Pyoverdine*</td>
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<td></td>
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*not measured. ^1Average of three independent cultures
Figure S3. Confirmation of PCN production by the *phzF2* mutant. Top: Extracted ion chromatograms for PCN for both the *phzF2* mutant (blue) and commercial standard (red). Bottom: MS/MS of PCN for both the *phzF2* mutant (blue) and commercial standard (red). The retention times, measured exact mass and MS/MS patterns are the same between PCN produced by the *phzF2* mutant and a commercial PCN standard.
Table S2. Metabolite Production by PAO1 and the *phzF2* mutant

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Calculated Mass</th>
<th>Measured Mass</th>
<th>ppm error</th>
<th>MPAO1 Peak Area(^1)</th>
<th>PhzF2 Peak Area(^1)</th>
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<td>PYO</td>
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<tr>
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</table>

*not measured. \(^1\)Average of three independent cultures
Figure S4. (A) 3-oxo-C12-HSL is detected by IMS as a spotted standard on ISP2 agar. Both PAO1 and PhzF2 produce metabolites corresponding to \( m/z \) 298. (B) The structures and exact masses for the [M+H]\(^+\) and [M+Na]\(^+\) forms of 3-oxo-C12-HSL are shown. (C) Extracted ion chromatograms for \( m/z \) 298.2013 and 320.1832 for 3-oxo-C12-HSL alone, PAO1 and PhzF2. (D) Mass spectra for 3-oxo-C12-HSL alone, PAO1 and PhzF2. The \( m/z \) values observed in PAO1 and PhzF2 samples do not correlate to 3-oxo-C12-HSL. They correlate to the quinolone C11:db UHQ ([M+H] = 298.2165). Expected ppm error is within 5 ppm for the settings used for mass spectrometry analysis.
Figure S5. Extracted ion chromatograms from LC-MS/MS analysis of PAO1 and the PhzF2 mutant. The area under the curve was calculated and fold changes of metabolite production were determined as displayed in Figure 2 of the main text.
Figure S6. (A) Inhibition of A. fumigatus Af293 by chemical complementation with rhamnolipids. The distribution of Rha-Rha-C10-C10 (Na⁺ salt) is represented in the IMS image. (B) The observed increase in surface area of mutants affecting the phenazine biosynthetic gene clusters is recapitulated. Optical images of PAO1 and the phzF2 mutant are shown after 4 days of growth on ISP2 media.
Figure S7. Approximately 12 quinolones were detected by IMS. All quinolones had similar distribution patterns.
**Figure S8.** (A) Extracted ion chromatogram for $m/z$ 260.1645 from PAO1 grown on ISP2 agar media. (B) MS analysis of peak at 26.5 minutes. (C) MS2 of $m/z$ 260.1659.
**Figure S9.** Extracted ion chromatograms of HHQ (A, m/z 244.1696) and HQNO (B, m/z 260.1645) produced by PAO1 (red) and the phzF2 mutant (blue). Production of both HHQ and HQNO is reduced in the phzF2 mutant.

**Table S3. Metabolite Production of PCN Complemented PAO1.**

<table>
<thead>
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<th>Metabolite</th>
<th>MPA01</th>
<th>MPA01 PCN</th>
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<td>8896510</td>
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*not measured. All values are averages of peak areas from three independent cultures.
Figure S10. Chemically complementing the phzF2 mutant with 1-HP, PYO, PCA, 5-MPCA, Phz (a mixture of the phenazines), PQS and 3-oxo-C12-HSL had no discernible effect on specialized metabolite production. 5-MPCA was not detected in the 5-MPCA complemented IMS image due to diffusion into the agar as it is water soluble. * denotes that fold-changes were not measured.

Table S4. Metabolite Production of Chemically Complemented phzF2 mutant

<table>
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<th>Metabolite</th>
<th>PhzF2</th>
<th>PhzF2 1-HP</th>
<th>PhzF2 PYO</th>
<th>PhzF2 PCA</th>
<th>PhzF2 5-MPCA*</th>
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*not measured. All values are averages of peak areas from three independent cultures.
Figure S11. IMS analysis of genetic complementation of the phzF2 mutant revealed some metabolite production was recovered although full complementation was not achieved with pJA09. Ara denotes addition of 0.2% L-arabinose to the culture medium to induce production of the phzF gene within the pJA09 construct. The phzF2 mutant is still producing PCN. However, due to the comparative visual nature of IMS, the higher production of PCN by phzF2 pJA09 and phzF2 pJA09 w/ Ara gives the visual impression that the phzF2 mutant is not producing PCN.
### Table S5. Metabolite Production of Genetically Complemented *phzF2* mutant

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<th>PhzF2 w/ Ara</th>
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<th>PhzF2 pJA09 w/ Ara</th>
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* not measured. All values are averages of peak areas from three independent cultures.
**Figure S12.** IMS analysis of the *phzF2* mutant complemented with the empty pHERD30T vector (pJA09 without *phzF*).
Figure S12. (A) IMS images of the genetic complement, m/z 238 and m/z 239. Although the images show possible production of 5-MPCA by the genetically complementing the *phzF2* mutant with pJA09, the mass spectrum shows a peak at m/z 238 (B) indicating that the m/z 239 observed is an isotope of m/z 238 and not 5-MPCA.
**Figure S13.** Chemical complementation of the interaction between *A. fumigatus* and the *phzF2* mutant by 1-HP, PYO and 5-MPCA causes small changes in triacetylfusarinine production by *A. fumigatus.*
Scheme S1. Synthesis of 5-MPCA

phenazine-1-carboxylic acid (PCA) → benzyl phenazine-1-carboxylate

BnCl, K₂CO₃, DMF (>95%)

5-methyl-phenazine-1-carboxylic acid (5-MPCA) → benzyl 5-methyl-phenazium-1-carboxylate

Pseudomonas fluorescens esterase (~58%)

MeOTf, CH₂Cl₂ (>95%)