Characterizing PqsE's enzymatic activity in *Pseudomonas aeruginosa* by Volatile Organic Compound analysis with GC×GC-TOFMS

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Abstract

Pseudomonas aeruginosa is an opportunistic pathogen commonly associated with severe respiratory infections in immunocompromised patients. The pathogenic behaviors of *P. aeruginosa* are controlled by a cell-to-cell communication system called quorum sensing (QS). Many genes activated by QS include those responsible for forming biofilms and producing virulence factors. Interestingly, several virulence factors produced by *P. aeruginosa* are regulated by an interaction between a receptor, RhlR and a hydrolase, PqsE. Thus, the PqsE-RhlR interaction has become a target for antibiotic development and further characterization. Comprehensive two-dimensional gas chromatography paired with time-of-flight mass spectrometry (GC×GC-TOFMS) was chosen for metabolomic analysis as it is known for its high sensitivity and improved peak capacity. With GC×GC-TOFMS, the volatile organic compound (VOC), acetophenone, was discovered and utilized to measure dose-dependent inhibition of PqsE's enzymatic activity *in vivo* by the small molecule inhibitor, Vorinostat. Furthermore, additional VOCs were identified that could add additional insight to PqsE's function.

QS and PqsE Enzyme Activity

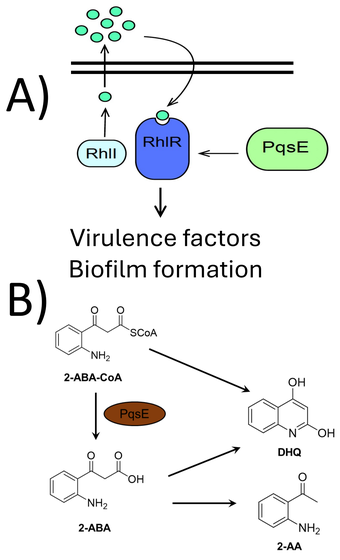


Figure 1. Quorum-sensing system of *P. aeruginosa*. (**A**) Diagram of the Rhl QS system. RhlI synthesizes signaling molecules that are received by RhlR. RhlR is responsible for gene transcription, and its interaction with PqsE is responsible for virulence factor production. (**B**) PqsE has known thioesterase activity in the Pqs QS system of P. aeruginosa, converting 2-ABA-CoA to 2-ABA; however, its role is redundant. No other substrates or products of PqsE enzyme activity have been reported to date.

Sampling Method

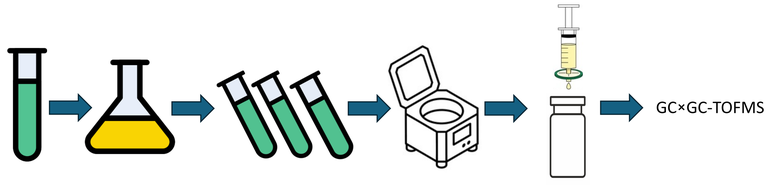
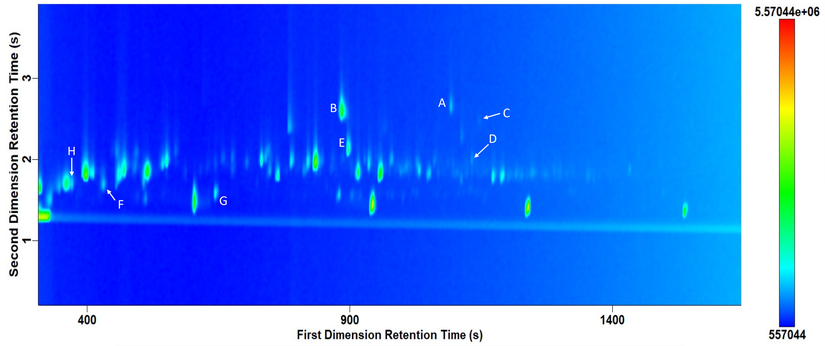


Figure 2. Supernatant sampling protocol. Strains were grown overnight and then diluted 100x. Diluted replicate cultures were grown for 8 hours and then centrifuged at 4000 rpm for 10 min. at 4 ˚C. Supernatants were filtered through a 0.22 µm filter into a headspace vial. Automatic sampling completed by a LECO L-PAL3 Autosampler. Ten replicates per strain

VOC Profile



Letter	Compound Name	F-ratio
A	Acetophenone	695.68
B	Benzaldehyde	60.58
C	Methyl benzoate	28.38
D	2-nonanone	55.32
E	2-ethylbutanol	27.84
F	2,4-dimethylfuran	18.84
G	2,4-dimethylheptene	15.5
H	2-methybutanal	19.37

Figure 3. Total Ion Current Contour Plot of a Wild Type (WT) sample. The WT strain has no mutations. Compounds were identified using Fisher Ratio analysis in ChromaTOF Tile. F-ratio threshold was 15.

Measuring Enzyme Activity

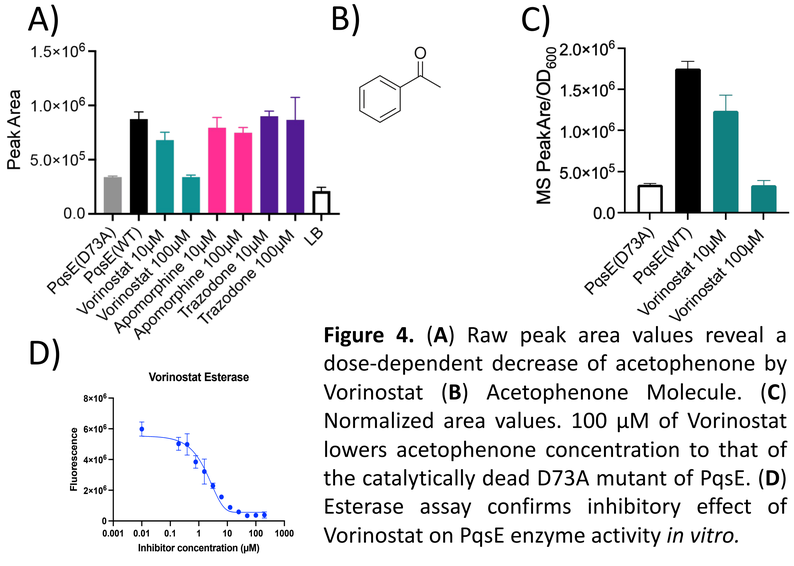


Figure 4. (**A**) Raw peak area values reveal a dose-dependent decrease of acetophenone by Vorinostat (**B**) Acetophenone Molecule. (**C**) Normalized area values. 100 µM of Vorinostat lowers acetophenone concentration to that of the catalytically dead D73A mutant of PqsE. (**D**) Esterase assay confirms inhibitory effect of Vorinostat on PqsE enzyme activity *in vitro.*

GC×GC-TOFMS and HS-SPME

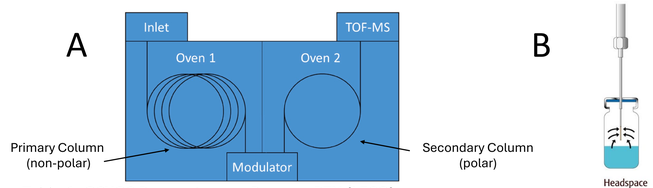


Table 1. GCXGC Parameters on Pegasus BTX (LECO)

Column	[1]D: Rxi-5MS (20 m × 0.18 mm ID × 0.18 µm d_f Restek Corporation) [2]D: Rxi-17Sil MS (3.7 m × 0.25 mm ID × 0.25 µm d_f Restek Corporation)
Modulation Period	4 s
Oven Temp. Program	40 °C (hold 2 min), ramp at 5 °C/min to 230 °C (hold 2 min)
Carrier Gas	Ultra high purity helium at 0.5 mL (1D column flow) with 30 mL/min (2D column flow) and a ratio of 4.5:1 sending to an auxiliary detector:mass spectrometer

Table 2. LECO L-PAL3 Autosampler

SPME Arrow Fiber	1.50 mm wide sleeve divinylbenzene/carbon wide range/polydimethylsiloxane (DVB/CWR/PDMS) fiber (Restek Corporation)
Incubation Period	2 min at 35 °C at 250 rpm
Extraction Period	5 min at 50 °C at 1000 rpm
Desorb time	2 min with an inlet temperature of 250 °C

Figure 5. GC×GC-TOFMS Diagram. (A) GC×GC utilizes two in-series capillary columns of different stationary phases connected by a modulator. The modulator traps narrow bands of primary column eluent and reinjects them into the secondary column. This refocusing leads to taller peaks which account for improved S/N ratio and sensitivity. (**B**). Headspace solid phase microextraction (HS-SPME) uses a coated fiber to concentrate volatiles and semi-volatiles present in the sample headspace.

Summary

- Eight VOCs identified that show statistically significant concentration differences between WT and D73A samples.
- The VOC acetophenone can be used to measure the enzymatic inhibition of PqsE *in vivo*.
- Vorinostat displayed a dose-dependent decrease in acetophenone levels.

Future Directions

- Optimizing the *in vivo assay* for a faster method with shorter run time.
- Improving the GC×GC and auto-sampler method to increase S/N for low-level analytes.
- Further screening of possible inhibitors.
- Streamline data processing method.

Acknowledgements

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Figure 5A: Carrillo, J.-C.; Shen, H.; Momin, F.; Kral, O.; Schnieder, H.; Kühn, S. GTL Synthetic Paraffin Oil Shows Low Liver and Tissue Retention Compared to Mineral Oil. *Food and Chemical Toxicology* **2022**, *159*, 112701. https://doi.org/10.1016/j.fct.2021.112701.
Figure 5B: https://www.palsystem.com/en/sample-prep-injection/micromethods/solid-phase-micro-extraction-spme/

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