Supporting Information

Spatiochemically Profiling Microbial Interactions with Membrane Scaffolded Desorption Electrospray Ionization-Ion Mobility- Imaging Mass Spectrometry and Unsupervised Segmentation

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Supplemental Tables and Figures

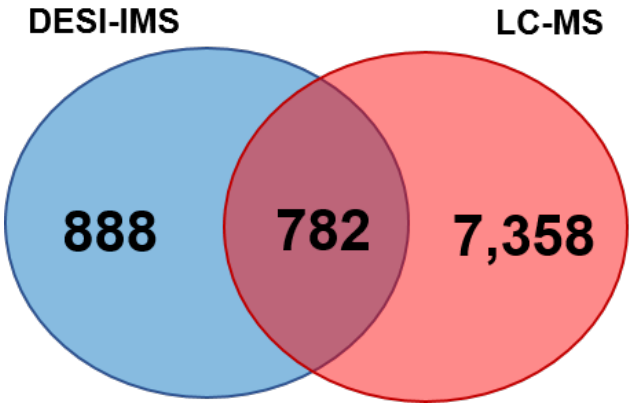

Figure S1. Venn Diagram showing the number of conserved and unique features between LC-MS and DESI-MS.

LC-MS acquisition settings:
Liquid chromatography-mass spectrometry (LC-MS) experiments were performed on a Waters Synapt G2 HDMS (Milford, MA, USA) mass spectrometer equipped with a Waters nanoAcquity UPLC system and autosampler (Milford, MA, USA). Metabolites were separated on a reverse phase 1 mm × 100 mm HSS T3 C_{18} column packed with 1.8-µm particles (Waters, Milford, MA, USA) held at 45°C. Liquid chromatography was performed using a 30-min gradient at a flow rate of 75 µL min^{-1} using mobile phase A (0.1% formic acid in H_2O) and mobile phase B (0.1% formic acid in ACN). The following elution gradient was used for analysis: 0 min, 99% A; 1 min, 99% A; 10 min, 40% A; 20 min, 1% A; 22 min, 1% A; 25 min, 99% A.

LC-MS analyses were run using high-resolution mode, with a capillary voltage of – 0.8 kV, source temperature at 100°C, sample cone voltage at 30 V, extraction cone voltage at 5 V, source gas flow of 400 mL min^{-1}, desolvation gas temperature of 325°C, and He cell flow of 180 mL min^{-1}. The data were acquired in negative ion mode from 50 to 1200 Da with a 1-s scan time; leucine enkephalin was used as the lock mass (*m/z* 554.262). All analytes were analyzed using MS^E with an energy ramp from 10 to 40 eV and an injection volume of 5 µL.

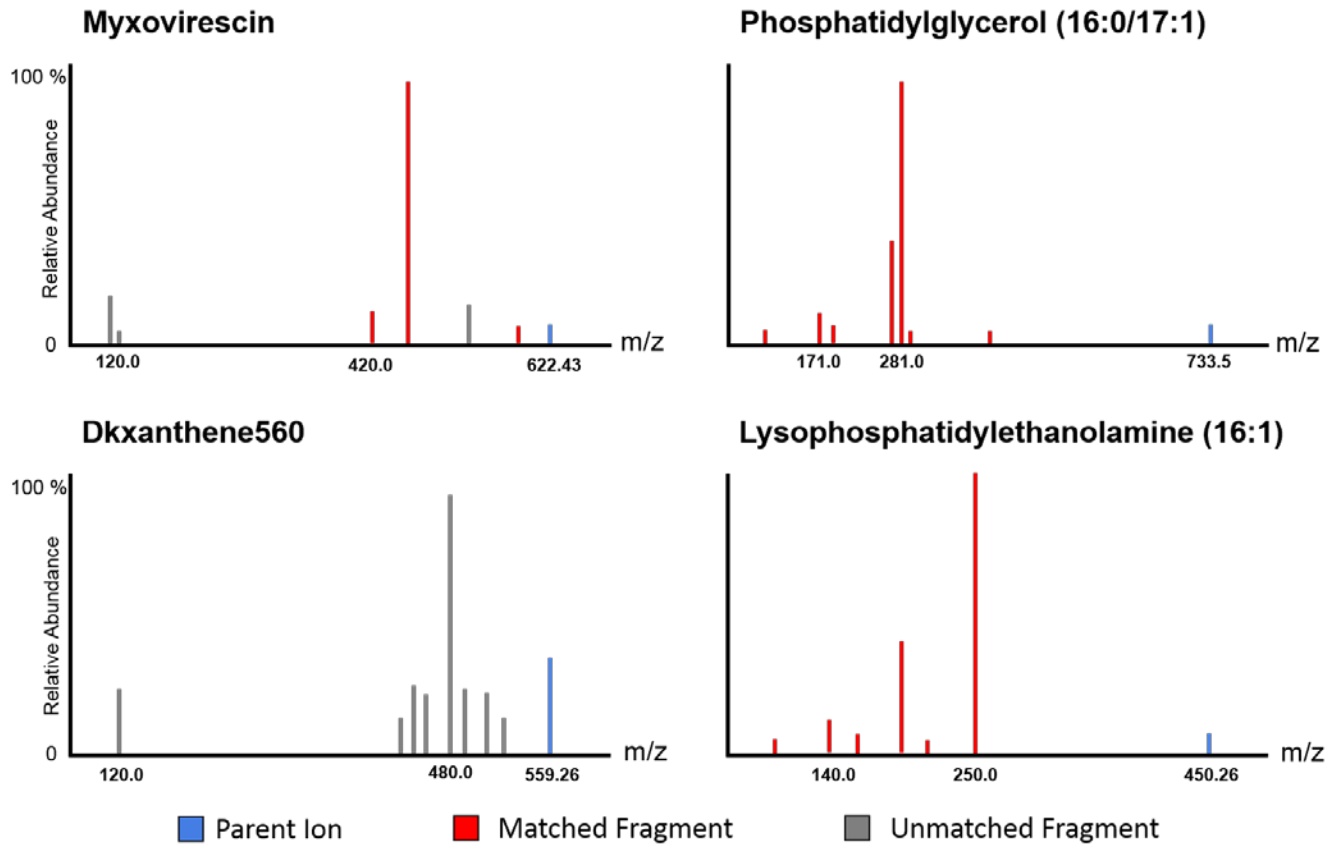

Figure S2. Fragmentation spectra of identified metabolites.

Table S1. Calculated values annotating sampling efficiency across methods (n=3).

Sample	Dried Agar	Imprinting	Microporous Membrane Scaffold
Average TIC	$4.30x10^9$	$9.84x10^9$	$1.75x10^9$
Significant Features (S/N ≥ 3)	193	340	355
Average Intensity*	$27x10^3 \pm 14x10^3$	$57x10^3 \pm 25x10^3$	$27x10^3 \pm 5x10^3$
Percent Covariance*	54.60%	44.10%	20.20%
Average S/N*	15.5 ± 10.6	110.3 ± 49.65	77.1 ± 15.0

* Calculated using Dkxanthene-534, Dkxanthene-560, Myxovirescin A, lyso-PE 16:1, and PG 16:0/17:1

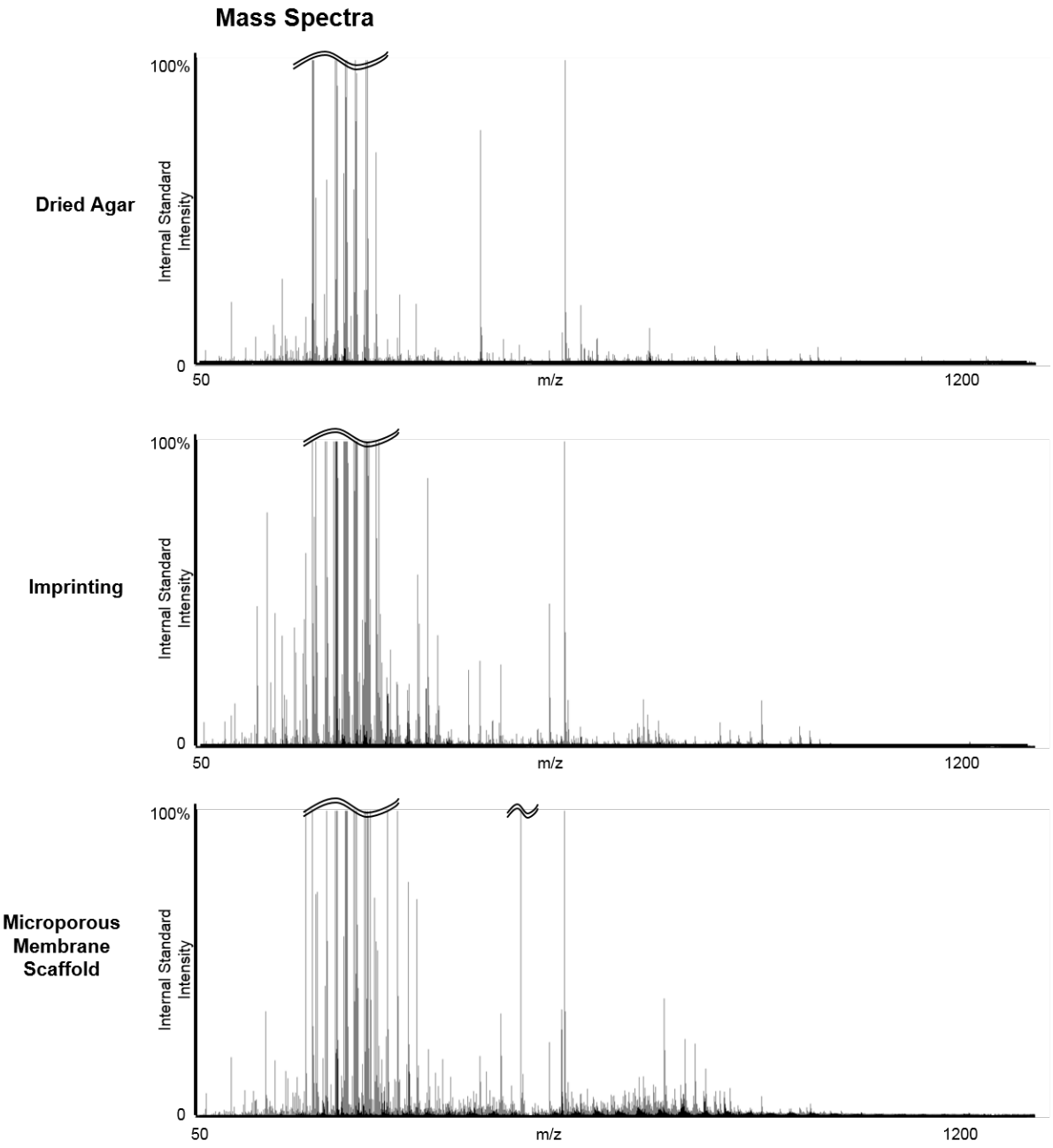

Figure S3. Average Mass Spectra of dried agar, imprinting, and microporous membrane scaffold sampling methods. Intensities are shown with the internal standard and lock mass leucine-enkephalin (m/z 554.26) intensity representing 100%.

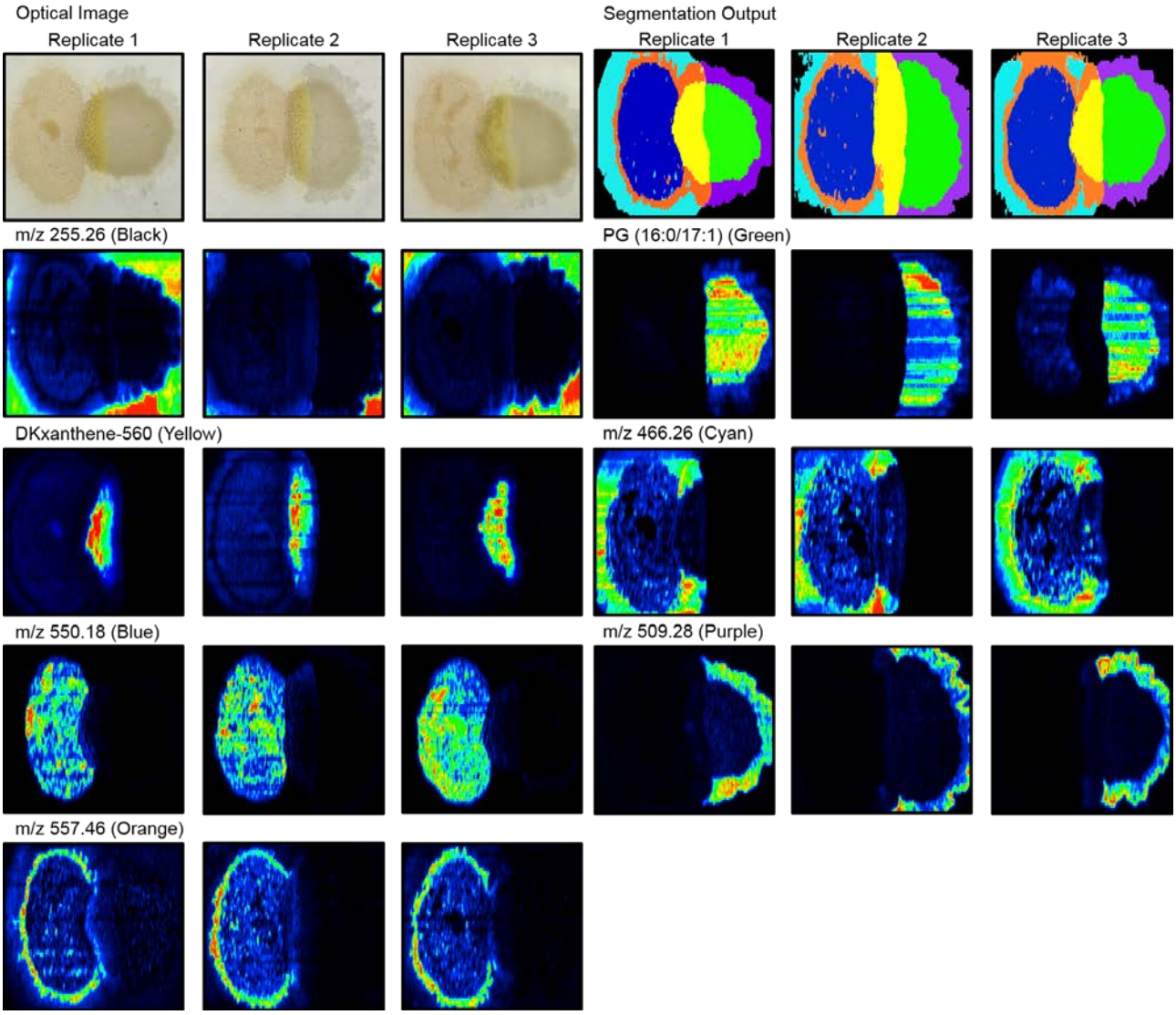

Figure S4. Biological replicates of unsupervised segmentation from MMS DESI-IMS. The ion image of the primary contributing feature to each segment is shown across replicates with the segment denoted in parentheses by color.

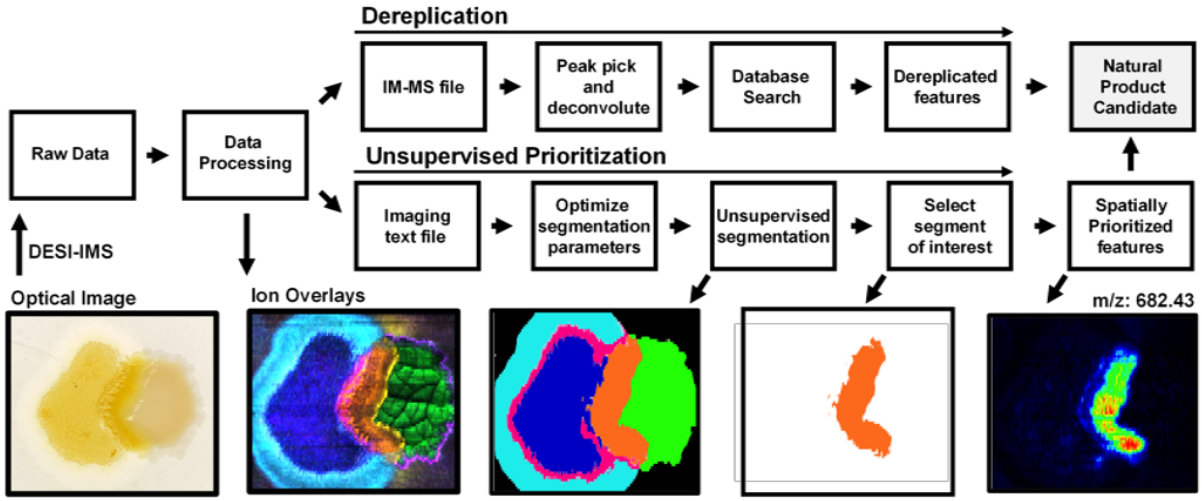

Figure S5. Natural product discovery workflow combining unsupervised segmentation and dereplication.

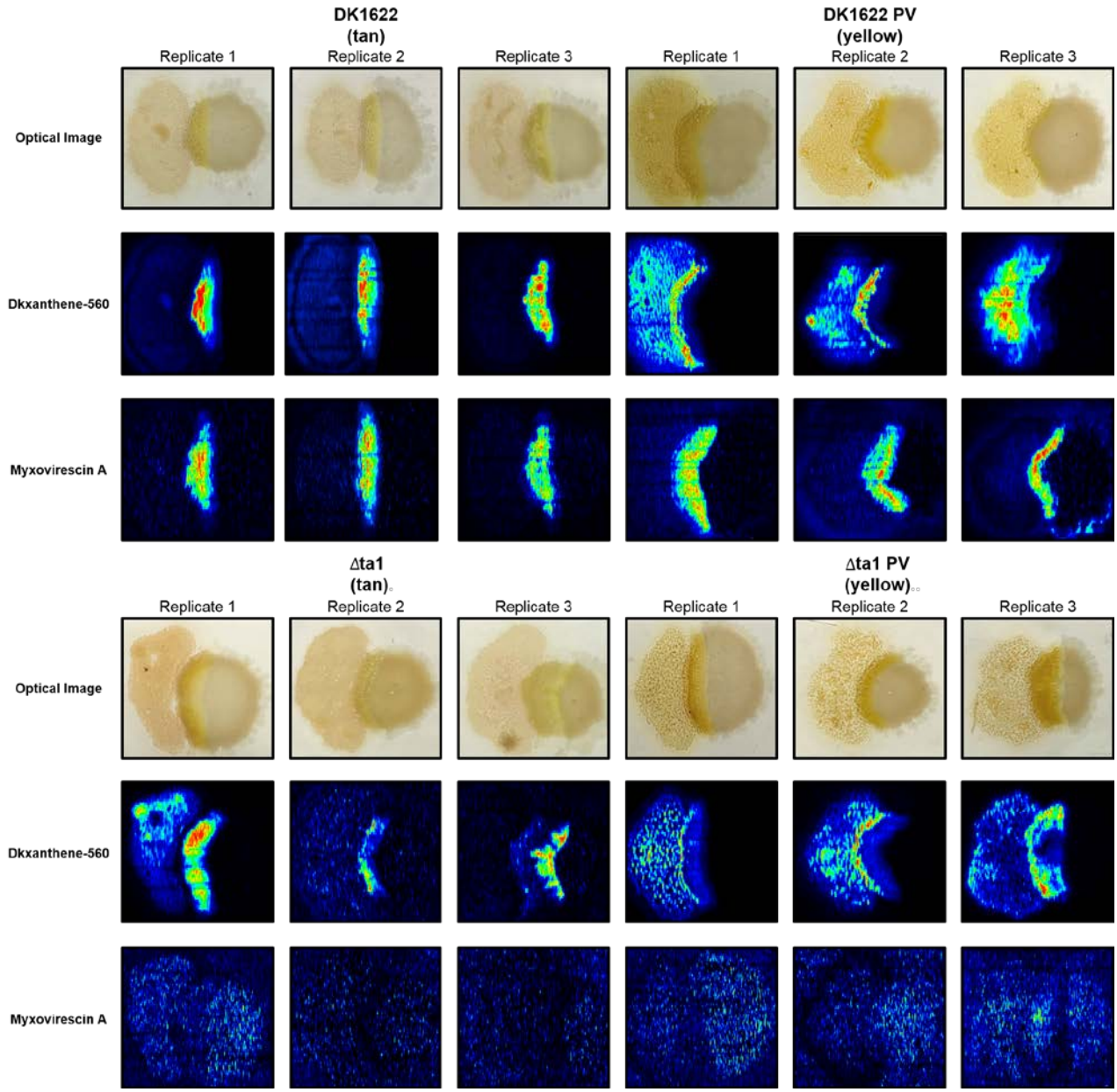

Figure S6. Production of reported natural products Myxovirescin A and DKxanthene-560 in biological replicates of WT and *M. xanthus Δta1* phase variants.

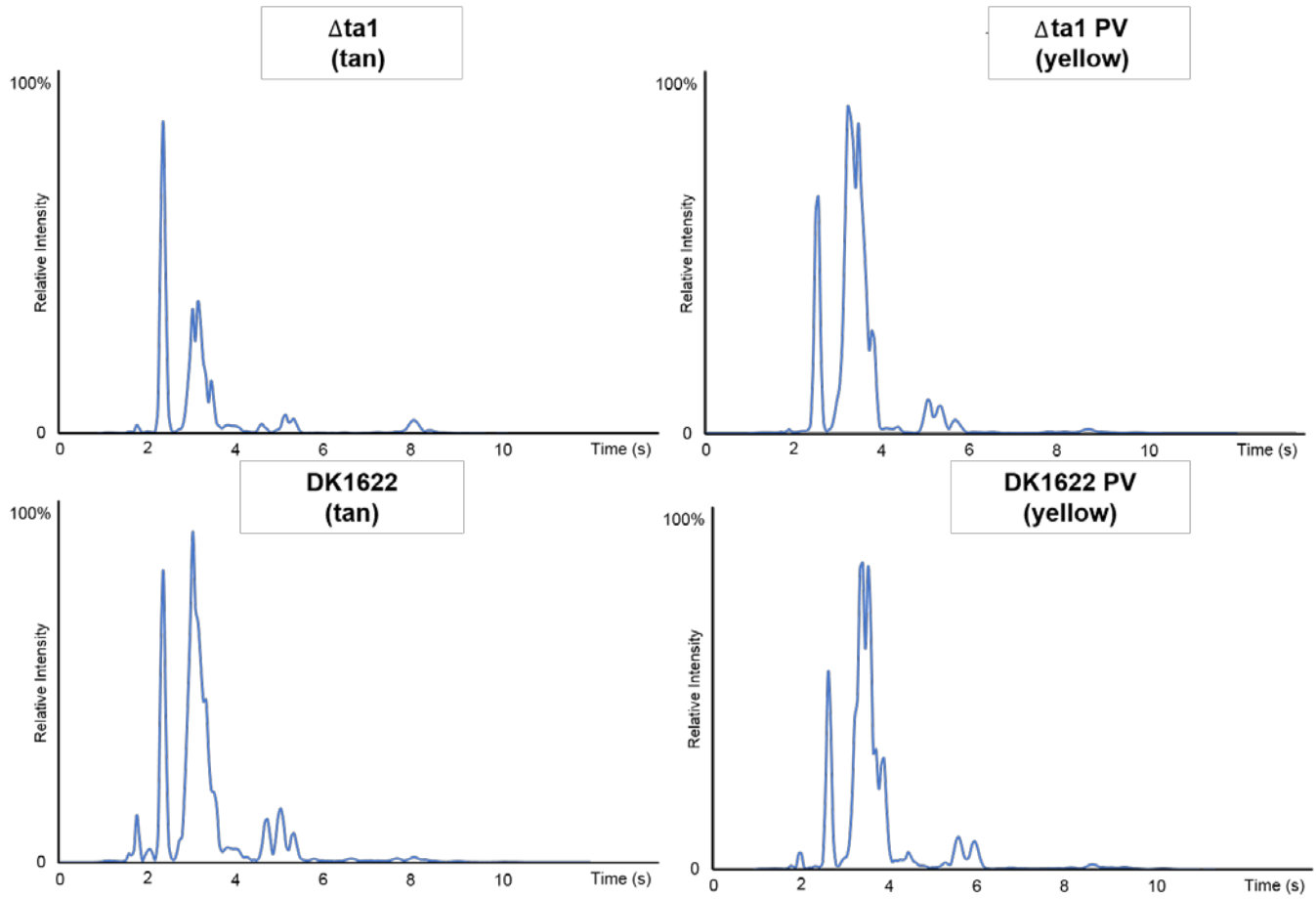

Figure S7. Drift time vs relative intensity of WT and *M. xanthus Δta1* phase variants.

Table S2. Observed features with regions resulting from unsupervised segmentation. Columns correspond to: "t-statistic" value denoting their significance in the region, standard deviation of "t-statistic" values across replicates (n=3), identification, molecular formula, adduct, mass accuracy in parts per million (ppm), and normalized intensity.