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Simplified molecular imaging analysis of excreted microbial metabolites using a benchtop MALDI-TOF system

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1. Overview

There has always been a high barrier to the application of MALDI imaging as this type of research has historically been the realm of high-end, high-cost MALDI-TOF instruments. There have also been challenges with the sample topography in imaging due to variable colony thickness. This work aims to demonstrate the utility of an affordable, entry-level linear benchtop MALDI-TOF mass spectrometer and its application to locate target biomolecules relevant to polymicrobial biofilms.

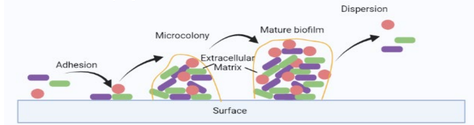

Figure 1: Schematic of Biofilm Formation

2. Introduction

Biofilms (Figure 1) are common throughout healthcare, industrial and environmental settings, with an estimated 65% of bacterial infections biofilm related [1]. Bacterial metabolites such as quorum sensing molecules produced by *Pseudomonas aeruginosa* (PA) have been validated as lung infection biomarkers. This is particularly relevant in Cystic Fibrosis (CF) [2]. Our aim was to use mass spectrometry (MS) approaches to better understand biomolecules produced by mono- and polymicrobial biofilms, changes that are relevant macroscopically and spatial distributions at the site of the biofilms microscopically. Here we show that accurate mass LESA MS metabolite and lipid profiling can be combined with spatial information from MALDI imaging to offer new insights into the behaviours of bacterial metabolites and lipids produced by biofilms.

Figure 2 Sample preparation for exo-metabolite analysis on Fleximass-DS MALDI target slides

3. Methods and Materials

Fleximass-DS MALDI target slides were lightly sanded to enhance agar adhesion. The MALDI target slide was loaded into a target mask and 500 µL of RPMI media poured on to each slide to create a thin agar layer over the MALDI target. Overnight cultures were used to inoculate discs on top of the agar-coated MALDI targets, or 6-well plate for LESA. After 24 hr incubation, the discs were removed (Figure 2) [3]. The MALDI targets were dried prior to applying 9-aminoacridine (9-AA) MALDI matrix using the iMLayer™ automated matrix sublimation device (Shimadzu, Figure 3). Imaging data was acquired in negative ion mode using a MALDI-8030 dual polarity MALDI-TOF mass spectrometer (Shimadzu, Figure 4) . 1:1 methanol: water (quorum sensing metabolites) or 2:1 chloroform: methanol (rhamnolipids) was used for LESA (Advion) MS and MSMS on an Orbitrap Exactive or Q-Exactive (Thermo) in both polarities.

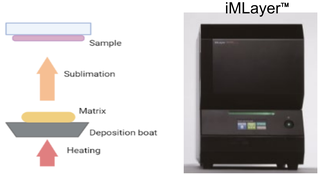

Figure 3 Schematic and image of the iMLayer™ sublimation device

-Dual mode MALDI-TOF
-200 Hz solid-state laser, 355 nm
-Load-lock chamber for fast sample introduction
-Benchtop design
-Quiet operation (<55 dB)

Figure 4 MALDI-8030 dual polarity benchtop mass spectrometer

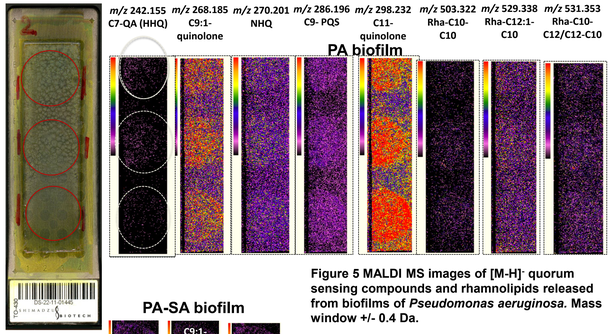

Figure 5 MALDI MS images of [M-H]⁻ quorum sensing compounds and rhamnolipids released from biofilms of *Pseudomonas aeruginosa*. Mass window +/- 0.4 Da.

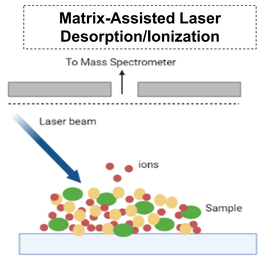

Figure 6 Schematic of MALDI

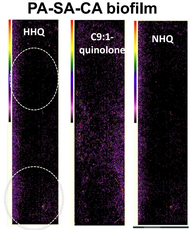

Figure 7 MALDI MS images of [M-H]⁻ quorum sensing compounds and rhamnolipids released from biofilms of *Pseudomonas aeruginosa* co-cultured with either *Staphylococcus aureus* (SA) and/or *Candida albicans* (CA). Mass window +/- 0.4 Da.

4. Results

MALDI Imaging offers insights as to the spatial distributions of metabolites (quorum sensing signalling molecules) and lipids (rhamnolipids) produced by biofilms of *Pseudomonas aeruginosa* (PA), see Figure 5. The spatial distributions of these change in the presence of other microorganisms, with many no longer detected, selected ion images are shown in Figure 7. Reductions agreed with LESA data, see Figure 9. Most of the compounds studied here were homogenously distributed beneath the site of biofilm growth and decreased in signal intensity in the presence of a competing microbe(s), Similar distributions have been reported previously from biofilms [4]. LESA-MS coupled to Orbitrap mass analysis provides complementary data in the form of high accurate mass, shown in Table 1, and MSMS assignments of the ions in Figure 10.

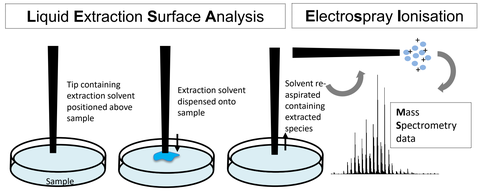

Figure 8 Schematic of LESA

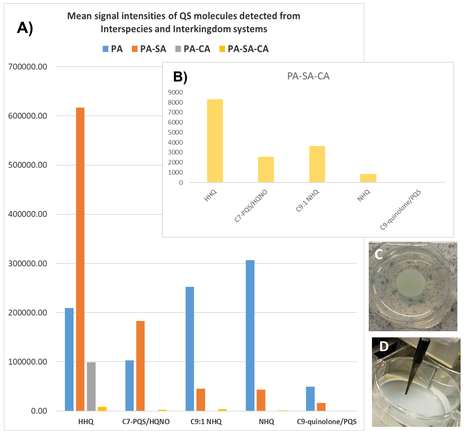

Figure 9 (A) Bar chart showing the mean signal intensity of QS molecules detected via LESA-MS in exo-metabolome analysis of interspecies biofilms. Mean values for PA-SA-CA, which were lower than other biofilms are shown in the inset in (B). Data is from nine repeats, three biological and three technical repeats; C and D showing sample preparation and sampling.

5. Conclusions

- MALDI Imaging allows visualisation of the spatial distribution of metabolites and lipids produced by microbial biofilms for the first time, showing homogenous distributions in PA.
- LESA provides complementary data allowing high accurate mass assignments and structural characterisation.
- Metabolites such as PA quorum sensing molecule HHQ were elevated in the presence of SA, whereas most metabolites were suppressed in the presence of CA. However, in MALDI images we saw a low distribution of HHQ when co-cultured with SA.

Detected m/z	ppm	Chemical Formula	Assignment	PA	PA-SA	PA-CA	PA-SA-CA
244.1696	2.2	$C_{16}H_{23}NO$	HHQ	*	*	*	*
260.1642	3.5	$C_{16}H_{23}NO_2$	HQNO	*	*	X	*
270.1849	3.3	$C_{17}H_{23}NO$	C9:1 quinolone	*	*	X	*
272.2007	2.6	$C_{16}H_{25}NO$	C9 quinolone	*	*	X	*
288.1955	3.1	$C_{16}H_{25}NO_2$	C9 PQS	*	*	X	X
300.232	2.3	$C_{20}H_{29}NO$	C11 quinolone	*	*	*	X
211.0863	3.8	$C_{13}H_{11}N_2O$	pyocyanin	*	*	*	X
475.2930	4.8	$C_{24}H_{44}O_9$	Rha-C8-C10	*			
503.3238	3.6	$C_{26}H_{52}NO_9$	Rha-C10-C10	*			
531.3546	2.4	$C_{26}H_{52}O_{13}$	Rha-C12-C10/C10-C12	*			
529.3399	4.2	$C_{28}H_{50}O_9$	Rha-C16:1-C10	*			

Table 1 Quorum sensing compounds and rhamnolipids released from biofilms of *Pseudomonas aeruginosa* (PA), and polymicrobial (interkingdom) systems[3], with *Staphylococcus aureus* (SA) and/or *Candida albicans* (CA) via LESA-MS.

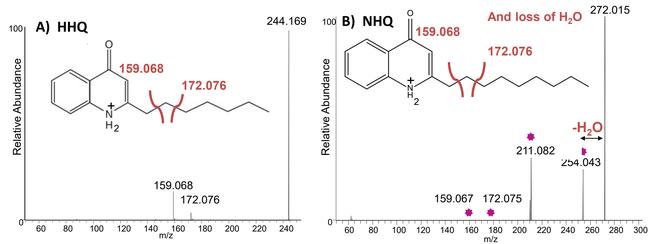

Figure 10 LESA MS/MS of quorum sensing compounds. Positive ion mode, [M+H]⁺ ions.

6. References

1. Jamal, M., et al., JCMA, 2018.
2. Barr, H.L., et al. ERJ, 2015.
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7. Acknowledgements

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