

Metabolite extraction from *E. coli* samples

15 mL from each replicate was collected and quenched according to the procedures developed by ¹. The collected cultures were quenched in 30 mL of 60% cold methanol (-48°C) and rapidly mixed, after which the quenched culture was centrifuged at 5000 xg and -9°C for 10 min. Following this, the supernatant was quickly removed and the bacterial pellets that remained were centrifuged for another 2 min and the remaining supernatant was removed. It was possible at this point to sample the quench supernatant to determine whether there had been any leakage of metabolites. The bacterial extraction was applied following the method in ¹. The bacterial pellets were suspended in 750 µL of 80% [v/v] methanol at -48°C, put into 2 mL tubes then liquid nitrogen was used to flash freeze. After this they were put on wet ice and when they were partially defrosted the samples were thoroughly vortexed for about 30 s. The cycle of freeze-thawing and vortexing was repeated twice more to ensure the maximum possible intracellular metabolites were extracted from within the cells. The suspensions were centrifuged at 13000 xg and -9°C for 5 min. The supernatants were collected and placed in clean 2 mL tubes then held on dry ice. The pellet had 750 µL of 80% methanol (-48°C) added to it and the entire process was repeated. The second extraction aliquot was mixed with the first one which was held on dry ice (which placed on the dry ice) and was subsequently thoroughly vortexed. These combined aliquots were lyophilized over-night and subsequently reconstituted in 100µL of methanol for LCMS analysis.

Ultra High Performance Liquid Chromatography - Mass Spectrometry (UHPLC-MS)

UHPLC-MS analysis was carried out on an Accela UHPLC autosampler system coupled to an electrospray LTQ-Orbitrap XL hybrid mass spectrometry system (ThermoFisher, Bremen, Germany). Analysis was carried out in negative ESI modes whilst each run was completely randomised to negate for any bias. A gradient type UHPLC method was used during each run as is previously described by ^{2,3}. 10 µL of the extract was injected onto a Hypersil GOLD UHPLC C₁₈ column (length 100mm, diameter 2.1 mm, particle size 1.9 µm, Thermo-Fisher Ltd. Hemel Hempsted, UK) held at a constant temperature of 50°C whilst a solvent flow rate of 400 µL/min⁻¹ was used to drive the chromatographic separation.

Xcaliber software (Thermo-Fisher Ltd. Hemel Hempsted, U.K.) was used as the operating system for the Thermo LTQ-Orbitrap XL MS system following the method described in ³.

Data processing was initiated by the conversion of the standard UHPLC raw files into the universal NetCDF format *via* the software conversion tool within Xcaliber. Subsequently, in-house peak deconvolution software containing the XCMS algorithm (<http://masspec.scripps.edu/xcms/xcms.php>) was used for peak picking as described previously^{3,4}. The output from this system resulted in a MS Excel based data matrix of mass spectral features with related accurate m/z and retention time pairs. Data from the internally pooled QC samples was then used to align for instrument drift and quality control (*via* application of an in-house Matlab script⁴). The data matrix was also signal corrected to remove peaks that crossed the 20% RSD threshold within QC samples across the analytical run. Normalisation of each peak within the samples was achieved using the mean peak area whilst putative identification of lipid features were performed applying the PUTMEDID-LCMS set of workflows as previously described⁵. Ambiguity arising from the same m/z ratio can lie within lipid identification due to differing points of unsaturation and multiple identifications. Multiple adducts of the same lipid can also occur due to the presence of different charged (composite) species (i.e. protonated and sodiated ions).

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