Extraction protocol

Metabolite samples were harvested in the mid-exponential phase in biological triplicates and technical duplicates. A Fast-Cooling method (Fu et al. 2015; Hollinshead et al. 2016) was used to quench the harvested cells. Briefly, ~15 mL culture (> 5 OD cells) was directly poured into 5 mL chilled M9 (without glucose) in a 50 ml falcon tube. The tube was then dipped in liquid nitrogen for 10 secs to bring down the sample temperature to 0^{o}C. To prevent the formation of ice crystals, the sample was vigorously agitated with the help of a digital thermometer. Samples were then immediately centrifuged at 0^{o}C, 7800 rpm for 7 min. The supernatant was discarded and the pellet was snap frozen in liquid nitrogen and stored at -80^{o}C until metabolite extraction was done.

For extraction of metabolites, the sample pellet was dissolved in 300 µL chilled (-80^{o}C) methanol and then transferred to a 2 mL tube. The samples were spiked with a fixed volume of ^{13}C labelled extracts as internal standard taken from the same batch. The samples were again snap frozen in liquid nitrogen followed by homogenization using a hand-held pestle (Sigma #Z359971) for 2 min. To this, 400 µL chilled methanol (-80^{o}C) and 300 µL chilled (-20^{o}C) chloroform was added and the tubes were incubated on ice for 2-3 min followed by vortexing. The sample tubes were then placed overnight on a Thermo-shaker maintained at 0^{o}C and 400 rpm. To the sample tubes, 500 µL of 2% ammonium hydroxide (4^{o}C) prepared in HPLC-grade water was added and incubated on ice for 10 min to enable phase separation. The tubes were then centrifuged at 4^{o}C, 13000 rpm for 15 min and the aqueous layer was collected in a chilled 1.5 mL tube. The samples were then completely dried in a vacuum concentrator and stored at -80^{o}C. Prior to analysis in LC-MS/MS, samples were reconstituted in chilled (-20^{o}C) 100 µL acetonitrile: buffered water (60:40, v/v), centrifuged at 4^{o}C for 10 min and the supernatant was transferred to pre-chilled glass vials. Buffered water consisted of 10 mM ammonium acetate, pH 9.23 adjusted with ammonium hydroxide prepared in HPLC- grade water (Teleki et al. 2015).