

Analytical Platform 11 (non-targeted LC-MS) was conducted by the Shulaev group at the Virginia Bioinformatics Institute. Extracts used for CE-ESI-MS analysis (described above), were also analyzed using hydrophilic interaction LC-MS/MS as previously described (Bajad et al., 2006). LC-MS/MS was performed on LC-10ADvp chromatographic system (Shimadzu, Columbia, MD) coupled to mass spectrometer. LC separation was performed on Phenomenex 250x2mm Luna 5 um aminopropyl column (Phenomenex, Torrance, CA) using gradient elution with 20 mM ammonium acetate + 20 mM ammonium hydroxide in 95:5 water:acetonitrile, pH 9.45 (Solvent A) and acetonitrile (Solvent B). The gradient profile was as follows: t = 0, 75% B; t = 15 min, 0% B; t = 38 min, 0% B; t = 40 min, 85% B; t = 50 min, 85% B. LC conditions were as follows: autosampler temperature 4 °C, column temperature 15 °C, injection volume 20 µL, and solvent flow rate 250 µL/min. Mass spectrometry analysis was performed on TSQ Quantum (Thermo Electron Corporation, San Jose, CA) triple quadrupole mass spectrometer. Column effluent was introduced into the electrospray ionization (ESI) ion source using fused silica capillary. Mass spectrometer was operated in a single reaction-monitoring (SRM) mode. Mass spectrometer conditions were as follows: ESI spray voltage at 3200 V in positive mode and 3000 V in negative mode, nitrogen sheath gas at 30 psi, nitrogen auxiliary gas at 10 psi, argon collision gas at 1.5 mTorr, ion transfer capillary temperature at 325°C. Scan time was 0.1 s for each SRM with a scan width of 1 nm/z. Metabolite concentrations were estimated relative to the concentration of the internal standard 4-fluoro-3-nitrobenzoic acid spiked at 25 µM.

Bajad SU, Lu W, Kimball EH, Yuan J, Peterson C, Rabinowitz JD (2006)
Separation and quantitation of water soluble cellular metabolites by hydrophilic interaction chromatography-tandem mass spectrometry. *J Chromatogr A* **1125**: 76-88