# Intact protein profiling and deconvolution of bacterial lysates on multiple mass spectrometers

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## INTRODUCTION:

Mass spectrometric analysis of intact protein bacterial lysates generates unique protein expression profiles for bacterial strain differentiation. The greater information content afforded to electrospray ionization makes LCMS preferable to MALDI when differentiating at a serovar or strain level. Advances in protein chromatography and LCMS compatible deconvolution software make it possible to generate reproducible accurate mass profiles specific to a given serovar. However, prior experiments have shown that many chromatographically resolved proteins are not being detected. In our current work we investigate whether the same chromatographic separation on higher resolving power and higher sensitivity instruments reveals lower abundant proteins that are currently being missed. In addition, differences in electrospray ionization sources being employed offer varying improvements in sensitivity.

## METHODS:

Salmonella proteins are extracted in 50:49:1 acetonitrile, water, formic acid using the **Barocycler Pressure Cycling Technology (Pressure BioSciences).** On-line separation of proteins is performed with two Kinetex (Phenomenex) 1.7 um C8 columns in series coupled to an Aquity UPLC (Waters). LCMS profiles are generated on three QTOF instruments, Premier (Waters), Synapt G2 (Waters), Maxis 4G (Bruker) and one Orbitrap Elite FT instrument (ThermoElectron). All data was deconvoluted and binned to accurate mass, retention time, and intensity profiles with a developmental version of ProteinTrawler (BioAnalyte) software.

## ABSTRACT:

LCMS data shows good protein level separation and reproducible deconvolution and binning of protein molecular weight and retention time profiles. Previous work has confirmed that we have chromatographic resolution of intact lysate proteins from 5 kDa to 100 kDa. We have also shown that ion suppression does not seem to be the major factor impacting the discrepancy between the number of proteins present in a bacterial lysate and the number proteins we actual detect. Our previous protein expression profiles were acquired on a QTOF Premier. More current instrumentation offers substantial increases in resolving power, sensitivity, and dynamic range. In addition, deconvolution algorithms that can process across an entire LCMS run have been expanded to be compatible with multiple data formats. Preliminary data has been acquired on a Synapt G2 QTOF and an Orbitrap. Data will also be collected on a Maxis 4G QTOF. Deconvoluted protein expression profiles will be compared across all platforms with the goal of determining if increased resolving power and sensitivity yield detection of lower abundant lysate proteins.