Mass Spectrometric Characterization of Phospholipids

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Following lipid extraction of bacterial cultures grown in the presence and absence of fatty acid, chromatographic separation will be performed using an ACQUITY-UPLC system (Waters, Milford, MA) equipped with autosampler, column oven, and binary pump for gradient elution. The UPLC is interfaced to a Quattro Micro (Waters) triple quadrupole mass spectrometer with electrospray ionization (ESI) source allowing UPLC-MS/MS analyses in both positive and negative ionization modes. Detailed characterization will use tandem mass spectrometry, or MS/MS, to generate unique fragmentation patterns for each phospholipid.

Initial studies (Fig. 1) have focused on obtaining molecular ions of the PLs by scanning over a narrow mass range (m/z 650–850) thought to encompass a variety of species likely to be present in the extracts. In addition, only ESI(-) data has been collected so far, whereas ESI(+) will allow more complete analysis since not all lipids ionize in negative mode. More detailed characterization is required to positively identify the new peaks observed in the chromatograms from fatty acid-exposed samples. This will be accomplished with a variety of mass spectrometry techniques.

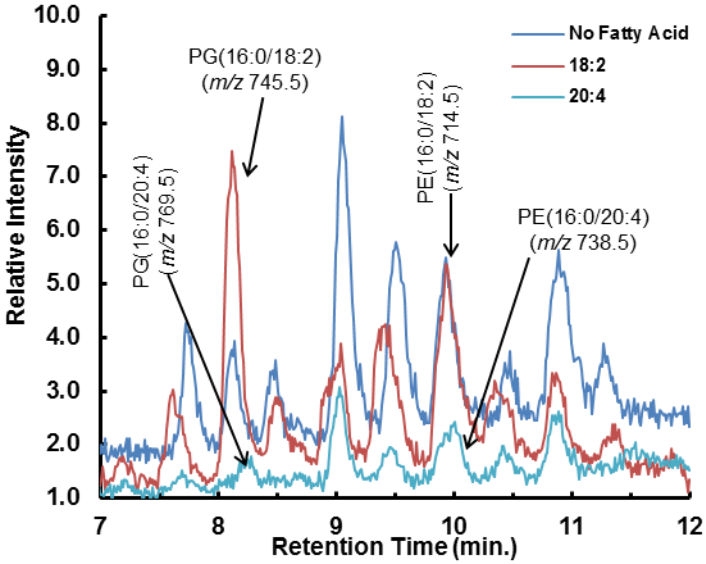

Figure 1. UPLC-MS chromatograms of phospholipids extracted from *P. aeruginosa* grown in the presence and absence of fatty acids. Lipids were extracted with Bligh and Dyer, dissolved in 15:85 [25mM ammonium acetate:methanol], and separated by gradient elution using a reversed-phase C8 column. Detection was by negative ESI quadrupole mass spectrometry. The chromatograms indicate quantitative and structural differences among phospholipid species between cultures. Arrows indicate peaks whose mass spectra correspond to the indicated phospholipid as predicted by the LIPID MAPS database based on molecular ion m/z.

We propose the following: (a) scanning a wider mass range (e.g. m/z 50 – 900) in order to observe specific headgroups and fatty acyl chains, (b) tandem MS to perform precursor ion and neutral loss scans, which provides class specificity (c) optimized collision energies for class-specific fragmentations, and (d) collision induced dissociation (CID) MS analyses to decipher lipid stereochemistry. The latter can be achieved using the relative ion intensity ratios for sn-1 and sn-2 cleaved fatty acids during collision experiments as previously described (39). The overall goal is to obtain an accurate inventory of the membrane PLs present in lipid isolates from bacterial cultures that have been grown with and without supplementation by exogenous fatty acids.