Proteomics of *Burkholderia* species using mass spectrometry

This project is being done in collaboration with Dr. Raina Maier's group in the Department of Soil, Water and Environmental Science at the University of Arizona http://ag.arizona.edu/SWES/people/cv/maier.htm

Burkholderia is a gram negative ubiquitous bacterium which can be found in variety of eco systems. There are number of species, some are beneficial to the environment and some those are more pathogenic and are recognized as possible bioterrorist agents. Among the genus, *B.pseudomallei* and *B.mallei* are the most pathogenic species. People have used various techniques to identify the virulence factors of these bacteria. Most of the virulence factors present in this organism are expressed in low abundance and normally are identified and studied by expressing them in *E. coli*. Here we demonstrate the use of mass spectrometry as an alternative technique to identify low abundant virulence factors from these bacteria without expressing them in *E. coli*.

Burkholderia cepacia which is an opportunistic pathogen to cystic fibrosis patients is being used as the model organism to develop methodologies for future experiments. *B.cepacia* has been recognized as a highly beneficial organism for the environment and for the plants. Very few proteomics studies have been carried out with *B.cepacia* due to the lack of a completed genome, making proteomics database searching a difficult task. Once the methodologies are optimized those methods will be used to analyze different proteome profiles of more pathogenic *B. pseudomallei* and *B.mallei* and also will compare it to the non pathogenic species *B. thailandensis*

Based on the whole cell proteins analysis, about 250 proteins were identified from LC-MS/MS data using database search algorithms. Ribosomal proteins, chaperons and cellular enzymes were the most abundant proteins identified in the whole cell proteome. Then proteins were extracted from three different cellular fractions as extra cellular, intra cellular and cell surface proteins. Fig. 1 shows the total number of proteins identified from the different cellular fractions using the SEQUEST database search algorithm. Total of 873 proteins were identified using all four fractions. As expected more proteins were identified in the intracellular fraction. Some proteins were seen in more than one fraction. Among the cell surface proteins we are able to identify several virulence factors of *B.cepacia* such as extracellular proteases, flagellin, porin, and phasing etc. as listed in the previous literature. This is a clear indication that mass spectrometry can be used to identify low abundant virulence factors. The fractionation procedures need to be optimized to increase the number of proteins identified.

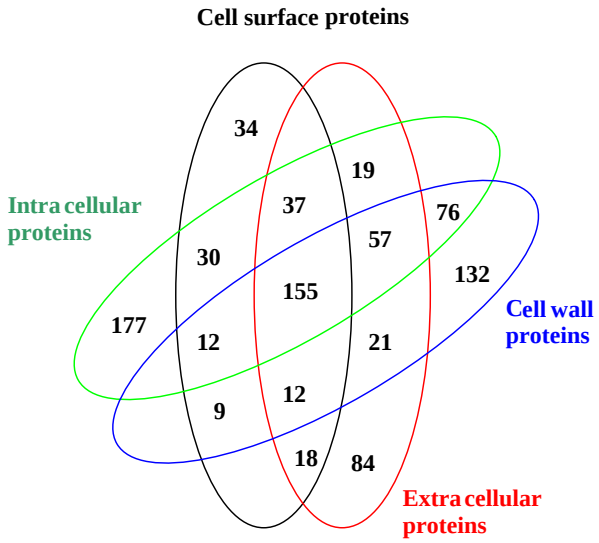

Fig.1. Total number of proteins identified from the different protein fractions using SEQUEST database search algorithm

Separate experiments were carried out without gel separation of the extracted proteins. Here the extracted proteins were tryptically digested and used strong cation exchange to separate the digested peptides prior to the LC-MS/MS analysis (MudPIT). Intracellular protein sample was analyzed using MudPIT. According to the search algorithms 434 proteins were identified with 297 proteins having two or more peptide matches. Based on the data, MudPIT gave more protein identifications than the gel separated samples.