OVERVIEW

We employed a "Label-free" mass spectrometry-based approach to analyze the proteomic profiles from two different types of Staphylococcus aureus (MSSA) and (MRSA) for the detection of potential markers for strain differentiations. The overlap of proteins identified between the strains was investigated.

A number of peptide bioinformatics were identified and quantitated for strain identification and for clinical diagnostics will be established in future studies.

INTRODUCTION

Methicillin-resistant Staphylococcus aureus (MRSA) has become increasingly prevalent worldwide, particularly with high incidences of its recovery from the 1990's in nursing homes and the community. Since staphylococcal infections are associated with high rates of mortality and morbidity, and have increased significantly during the 1990's in England and Wales, causing huge burden for the public health, with estimated costs of £15 million per year. This increase coincides with the emergence of multi-drug resistance among S. aureus with increased significantly during the 1990's in England and Wales causing a huge burden for public health, with incidences of its recovery from the 1960's in nursing homes.

METHODS

Sample preparation

1. Staphylococcus aureus strains H591 and D547 were cultured for 24 hours at 37°C on Columbia Blood Agar plates.
2. Cells were harvested and the proteins extracted by a combination of freeze-thawing and mechanical disruption as described previously for Staphylococcus aureus [2].
3. Protein concentrations of all extracts were adjusted to give a final concentration of 6 mg/mL.
4. 30 μL of each sample were loaded into a Waters micro pressure (Hewlett Packard) 1,500 μl/m and subjected to a mini electrophoresis prior to analysis.

LC CONDITIONS

Waters nanoACQUITY™ UPLC System

Column: Tryptic digest, Synapt.G2 C18 (130μm x 20mm, 5μm particle size)

Analytical column: RPH18 (75μm x 100mm, 1.7 μm particle size)

Gradient: H20/MeCN/formic acid at 300μL/min

METHODS

The low energy data are used for the quantification of the differentially expressed proteins. The high energy data are used for the identification of the unknown proteins.

Data processing

Databases were screened and processed using Waters Protein Expression System software. In order to search against a comprehensive database, the proteins were searched against the National Center for Biotechnology Information (NCBI) database, the Staphylococcus aureus database, and the Staphylococcus aureus database.

RESULTS

As expected the isolates showed a high degree of similarity in their proteomic profiles since they belong to the same species and share 90-100% DNA sequence homology. Figure 1 shows low energy (300μl/m) spectra of the protein ions detected in the differentially expressed proteins.

Figure 1 shows the overlap of 10 proteins detected in the low energy spectra of the Staphylococcus aureus strain and the high energy spectra of the Staphylococcus aureus strain.

Figure 2 highlights the complexity of the data as a low energy and a time aligned high energy spectra are displayed from an MESA replicate at 24.3 μs. Several precursor ions are present in the low energy spectrum and the aligned energy spectrum is complex.

Figure 3 shows the overlap of four strains from all three replicates for each strain.

Figure 4 shows the overlap of all four strains from all three replicates for each strain.

Table 1 shows an overview of the proteins detected from their characteristic peptides. Most represent key functions of the cell and includes such proteins as: glycerol-3-phosphate dehydrogenase, protein synthesis e.g. DNA binding protein (H3), Protein folding and cell signaling e.g. 3-phosphoglycerate kinase.

CONCLUSIONS

A total of 154 proteins were identified and quantitated between the two groups. The results are consistent with previous studies involving staphylococcal pathogens and may lead to the emergence and similar proteins.

FUTURE STUDIES

Future studies involving the characterization of staphylococcal proteins and the development of a microfluidic chip for the detection of potential markers for strain differentiation will be established in future studies.