Disclosure of Interest: None Declared

P1530

PROTEOME-WIDE SRM ANALYSIS OF IN STREPTOCOCCUS PYOGENES VIRULENCE MECHANISMS

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Background: *Streptococcus pyogenes* (group A streptococcus) is a gram-positive bacterium that is responsible for a wide range of human diseases worldwide, including both self-limiting localized and life-threatening systemic infections. In this study genome-wide Selected Reaction Monitoring (SRM) targeted proteomics is used to characterize differences between strains displaying a low and high grade of virulence in a mouse model.
Methods: Intracellular proteins, surface digestions and culture filtrate extracts of exponential and stationary phase bacteria were analyzed with a triple quadrupole mass spectrometer operating in SRM mode. This based on a genome-wide SRM assay repository generated by LC-MS/MS experiments and synthetic peptides. To address differences in host protein binding capability, eluates of bound plasma proteins were analyzed by LC-MS/MS.
Results: Preliminary results show that the major differences between the strains are differential abundance of proven virulence factors and hypothetical proteins. Additionally the genomic mutations are confirmed using the SRM technology. The virulent strain also displayed an elevated capability of binding human host proteins not seen in the low-grade virulent strain.
Conclusion: We are generating a comprehensive topological protein abundance map of *S. pyogenes* to distingue differences between high and low virulent strains.

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P1531

IDENTIFICATION OF IMMUNOREACTIVE PROTEINS FOR MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS DETECTION

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Background: Johne's disease is a *Mycobacterium avium* subsp. *paratuberculosis*(MAP)-caused chronic enteritis of ruminants associated with enormous worldwide economic losses for the dairy cow- and goat-rearing industries. Eradication programs and the management limitations for this disease have been hampered by the lack of simple and specific diagnostic tests for detecting the disease in subclinically infected (infected but symptom-free) animals. MAP is ubiquitous and it can be found in soil and water of infected farms, but actually there is no available method to find it. Aim of this project is to find, with proteomics, potential target proteins of MAP surface or secreted by MAP useful for developing a biosensor for its detection.
Methods: Two different protein solubilization methods have been developed. The first one was developed using both freeze thaw cycles and bead beating and the second one using French press cycles and strong detergents for pellet solubilization.
Results: According with obtained results it is possible to say that both are good methods for MAP protein extraction but bead beating is more convenient because of its higher protein recovery rate. This result is probably due to a better cell disruption obtained with the bead beating procedure.
Moreover three immunogenic proteins were found with 2D immunoblotting, one of them is really interesting because of its similarity with a previously described immunogenic protein, the MAP2121c that is a 35 kDa membrane protein with a strong immunogenic activity and specificity.
Conclusion: Obtained results demonstrate, according with previous findings, that, because of its abundance and MAP specificity, MAP2121c can be used as possible candidate for developing a biosensor.

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