Poster 21

BIOMARKERS FOR BOVINE MASTITIS USING SURFACE ENHANCED LASER DESORPTION-IONISATION TIME OF FLIGHT MASS SPECTROMETRY

Eckersall PD[1], Hogarth, CJ[2]
[1]*Division of Animal Production & Public Health, University of Glasgow, Glasgow, Scotland* [2]*Ciphergen Biosystems Ltd, Guildford, Surrey, UK*

Introduction

Recent studies have shown that during bovine mastitis, acute phase proteins are produced by the mammary gland and are secreted in milk. However, there are likely to be other changes to the proteins in milk during the development of the disease which could be exploited as biomarkers for mastitis. Proteomic studies seek to examine the full complement of protein expression in a cell, tissue or fluid under a given set of physiological conditions. Previously, proteomic approaches combining two-dimensional gel electrophoresis (2-DE) and mass spectrometry have been used for the analysis of bovine milk. Low molecular weight proteins and peptides are notoriously difficult to analyse in conventional 2-DE based proteomic approaches. Surface enhanced laser desorption-ionisation time of flight (SELDI-TOF) mass spectrometry provides a methodology to extend the examination of the milk proteome to include these low molecular weight proteins.

Material and Methods

In the current study SELDI-TOF mass spectrometry was used to examine differentially expressed proteins in bovine milk from quarters of cows in an experimental model of *Staphylococcus aureus* induced mastitis. Milk samples were collected from infected quarters of 6 cows 42-48 hours after inoculation with *S aureus* and were compared to the milk from opposite, healthy, quarters in the same cows that had been inoculated with sterile saline. Following centrifugation to remove lipid and cellular material, 2 µl of the milk samples were applied to each individual spot of an 8-spot CM10 (weak cation exchange) array and incubated for 1h at room temperature. The arrays were then washed (3 x 5min) in a 100mM Na Acetate buffer, pH4 and left to dry at room temperature. After drying, 0.5 ml of saturated sinapinic acid (in 50% acetonitrile and 0.5% TFA) was applied to each spot twice, prior to final air-drying for 10 minutes. Arrays were analysed by SELDI-TOF mass spectrometry on a Series 4000 instrument (Ciphergen Biosystems Ltd).

Results

Comparison of the the SELDI-TOF mass spectrograms between milk samples from healthy and mastitic quarters in the same cow showed a clear increase in the number and peak heights for low molecular weight peptides (mass/charge (M/Z) ratio <20k). In total 63 statistically significant (p-value < 0.05) protein biomarkers were identified as being able to discriminate between mastitic and healthy milk. Of these, 44 were of low molecular weight).

Discussion

This preliminary study has shown that these is a considerable increase in the number and concentration of low molecular weight proteins and peptides in milk during the first 48 hours of bovine mastitis. Identification of these potential biomarkers should be undertaken to establish whether these are related to the acute phase proteins that are known to be secreted in milk during this condition or whether they are the result of protease action on milk proteins.

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