BRIEF COMMUNICATION: Use of cDNA microarrays to investigate gene regulation in mastitis as a result of *Escherichia coli* infection in the bovine mammary gland

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INTRODUCTION

Bovine mastitis is the result of an inflammatory event in the mammary gland usually caused by a variety of bacteria in the udder and is one of the three major causes of disease in the Dairy industry world wide. The cost of mastitis in New Zealand is estimated at NZ$180 million for the industry and (DairyInsight, 2006).

Around parturition and during early lactation *Escherichia coli* mastitis occurs with a divergent range of clinical symptoms leading to several cow deaths each year. The host's immune reaction to *E. coli* mastitis is an important factor influencing the outcome of the disease. In order to obtain a greater insight into the host's immune reaction, cDNA microarray analysis has been performed. Applying an appropriate pipeline of normalization, clustering and pathway analysis of genes significantly differently expressed between diseased and non diseased state, can greatly assist in the identification of key genes and pathways involved in the cow's innate immune defense system. Ultimately this information could lead to the development of new strategies to combat mastitis.

MATERIALS AND METHODS

Three separate quarters of four udders were inoculated with *E. coli* for 6, 12 and 24 hours. Experimental design has been reported by Petzl *et al.* (2008). Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The RNA was labelled with reactive forms of Cy3 and Cy5 dyes (Ambion amino allyl cDNA labelling kit). RNA from infected and non-infected quarters from the same animal were hybridised to 24 cDNA microarray slides, developed by AgResearch (Singh *et al.,* 2004), in a humidified chamber at 50°C for between 16 and 24 hours. Dye swap was used as technical replication. A Genepix scanner and software (Axon instruments, Inc. CA, USA) was used to read the slides. Microarray data was then normalized for each individual slide as described by Baird *et al.* (2004) and the dye bias effect removed as described in Dabney *et al.* (2007).

RESULTS AND DISCUSSION

Gene expression profiles in the infected quarters of the udder were compared with the control quarter. A dataset containing 2,780 ESTs that showed a fold change of more than one and a half ($P < 0.05$) at one or more time points was analysed using a short time expression miner (STEM) (Ernst *et al.,* 2006) to identify unique expression time profiles of different gene sets. A total of 1,391 ESTs were assigned to eight significant different profiles. Four profiles, up regulated at 24 hours and four down regulated. Of these, 689 ESTs with known mRNA SwissProt hits were submitted to BioRag (www.biorag.org) for pathway analysis of each individual profile (Pandey *et al.,* 2004). Four Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (http://www.genome.ad.jp/kegg/) with ten or more ESTs up or down regulated were identified (see Table 1).

TABLE 1: Number of ESTs that were up or down regulated at 24 hours under an *E. coli* mastitis challenge in the different STEM profiles identified in KEGG pathways. STEM = short-term expression miner; MAPK = Mitogen-activated protein kinase; KEGG = Kyoto Encyclopedia of Genes and Genomes.

KEGG pathway	Up regulated genes					Down regulated genes					Total number of genes in pathway
	STEM profile number				Total	STEM profile number				Total	
	18	29	40	42		9	11	26	34		
MAPK signaling pathway	5	2	2	2	11			2		2	13
Antigen processing and presentation	3	1	3		7			5		5	12
Toll-like receptor signaling pathway	4	1	1	2	8			2	1	3	11
Leukocyte trans endothelial migration	4		4		8		1	1		2	10

Clustering of genes has the potential to reveal meaningful biological patterns. Not all clustering approaches take into consideration the sequential nature of time series (Ernst *et al.,* 2005). STEM is specifically designed to analyse short time expression series. Each gene is assigned to one of the temporal expression profiles and the enrichment of genes in each profile is computed to determine profile significance (Ernst *et al.,* 2006). ESTs in the same profile share a unique temporal expression pattern and are therefore highly likely to be co-regulated.

In this experiment the ESTs, TLR4, IRAK1 and AP-1 (Activator Protein-1) were identified to be part of a unique expression profile. The pathway analysis identified these ESTs as part of the Toll-like receptor (TLR)-signalling pathway. Goldammer *et al*. (2004) reported a strong increase of TLR4 mRNA in mastitis. Through the transcription factors NFκB and AP-1 it induces transcription of cytokines and β-defensins as a result of lipopolysaccharide (LPS) stimulation (Vora *et al.,* 2004). LPS is recognized as a pathogen-associated molecular pattern (PAMP) by TLR in an *E. coli* infection (Kawai *et al.,* 2006). There is a correlation between TLR4 and the β-defensin BNBD5 in cases of *S. aureus* mastitis (Goldammer *et al*., 2004). Defensins are known to be regulated by TLR4 signalling through NFkB regulation. However, Yang *et al.* reported that NFkB is essential but not the major switch for BNBD5 regulation of mammary epithelial cells challenged with *E. coli* (Yang *et al.,* 2006). Analysis of the cDNA microarrays suggests the co-regulation of TLR4 and AP-1. Wehkamp *et al.* (2004) reported that functional binding sites of NFkB and AP-1 in human β-defensins are required for the induction of β-defensins through *E. coli*. Therefore further investigation into the role of AP-1 in β-defensin regulation such as BNBD5 in *E. coli* bovine mastitis is warranted and will be performed in the future.

The use of publicly available bioinformatics tools has lead to the identification of potential candidate genes associated with the innate immune response of mammary epithelial cells challenged with E .*coli,* in line with existing biological knowledge. With further research, this may lead to the development of new strategies to combat mastitis.

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