Cross-talk between bovine mammary quarters during *E. coli* and *Staph. aureus* infection

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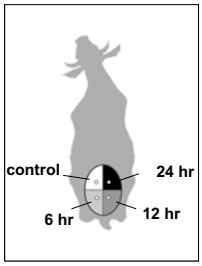
In vivo infection study

- 12 Holsteins in middle of first lactation.
- Individual udder quarters sequentially infected with:
  - 500 cfu *E. coli*
  - 10,000 cfu *S. aureus*
- 48 samples
  - 4 animals infected with *E. coli*
    - Quarters infected for 0, 6, 12 & 24 hours
  - 4 animals infected with *S. aureus*
    - Quarters infected for 0, 6, 12 & 24 hours
  - 4 animals infected with *S. aureus*
    - Quarters infected for 0, 12 & 72 hours
- ARK-Genomics Bov20K cDNA microarray (A-MEXP-1402)
- Two colour
- Common reference design
  - Multifactorial analysis
  - Reference = Mixed pool of all RNA samples
  - Cy3 = common reference sample
  - 48 slides



In vivo infection study: time course

- Very few differentially expressed genes (FDR<0.05; fold change >2) identified in samples except 24hr *E. coli* infection time point, even though clinical signs were detected at 12 hours post *E. coli* infection.

Hours	*E. coli*	*S. aureus*
6	0	1
12	6	4
24	1048	0
72	ND	0

(More genes were differentially expressed at P<0.05)

- Reasons?
  - Tissue samples collected: considerable variation in particularly *S.aureus*
  - Base-line provided by control quarters

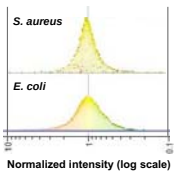
Genes differentially expressed

(Compared to control quarters)

E. coli 12 hr:	Fold change	*S. aureus* 6hr	Fold change
C0882029	↓2.1	AJ813772	↓2.6
C-C chemokine ligand 2	↑3.9	AJ819694	↑2.2
R3H domain containing	↑3.3	BF775518	↑3.7
BTG family member 2	↑3.0	LBP	↑4.8
Tribbles homolog	↑2.4	SOD2	↑5.9
Chr 17 ORF	↑ 2.3		
24 hr top:			
calgranulin A	↑21.9		
calgranulin C	↑19.8		
Lipin	↓5.4		
WIPI1	↓5.2		

Control quarters

- Uninfected control quarters:
  - Bacteriologically negative
  - No up-regulation of TLR2, TLR4 or β-defensins
  - However, SCCs increased during both infection types
  - (Petzl et al., 2008)



Log intensity plots (sample/reference) for uninfected quarters

Normal distribution around 1

***E. coli* broader distribution. *S. aureus* has greater similarity to the reference sample**

Comparison of the transcriptome of *S. aureus* and *E. coli* infections "control" quarters

- 255 clones (FDR ≤ 0.05, fold difference ≥ 2)
  - **212 annotated**
  - **187 genes**
- 156 clones (110 genes) *E. coli* > *S. aureus*
- 99 clones (77 genes) *S. aureus* > *E. coli*

Database for Annotation, Visualization & Integrated Discovery (DAVID) analysis

- Human RefSeq accession numbers were assigned where poss.
- Gene Ontology (GO) Biological Process terms:

- Enrichment of terms in order of frequency:
  - **Cellular component organization & biogenesis**
  - **Response to stress**
  - **Biosynthetic process**
  - **Cell development**
  - **Nitrogen compound metabolic process**
  - **Death (cell death)**
  - **Anatomical structure development**
  - **Cell activation**

Top differentially expressed genes

E. coli>S.aureus	Fold diff	*S. aureus>E. coli*	Fold diff
FK506 binding protein 5	6.56	EST	4.56
RNA terminal phosphate cyclase-like	5.89	myosin IXB	4.03
solute carrier family 38, member 7	5.35	ATP-binding cassette, sub-family G (WHITE), member 2	3.48
metallothionein 2A	5.24	EST	3.38
metallothionein 1A	4.60	isocitrate dehydrogenase 1 (NADP+), soluble	3.36
syntrophin, alpha 1	4.49	thymidylate synthetase	3.35
sml nucleolar RNA, C/D box 65, non-coding RNA	4.43	SUMO1/sentrin/SMT3 specific peptidase 2	3.32
solute carrier family 7	4.39	chemokine (C-X-C motif) ligand 12	3.23
leucine rich repeat neuronal 3	4.32	EST	3.18
sal-like 2 (Drosophila)	4.24	fasciculation and elongation protein zeta 1 (zygin I)	3.13
WD repeat domain, PI interacting 1	3.82	transducin-like enhancer of split 4 (E(sp1) homolog, Drosophila)	2.94
lipocalin 2 (oncogene 24p3)	3.76	EST	2.92
angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	3.73	leucine rich repeat containing 4C	2.92
		laminin, gamma 2	2.91
		protein phosphatase 2 (formerly 2A), regulatory subunit B, gamma isoform	2.84

Genes expressed at higher levels in *E. coli* infection control quarters

- **Metallothioneins (antioxidants & bind metals preventing up-take by *E. coli*)**
- **Lipocalin (binds *E. coli* siderophores to prevent iron up-take by *E. coli*)**
- **Lipopolysaccharide binding protein (involved in LPS/TLR4/CD14 signalling)**
- **FK506 binding protein 5 (activates NF-κB)**
- **Conclusion: *E. coli* infection of neighbouring quarters triggers a response in the control quarters**

Are the control quarters responding to *S. aureus* infection of neighbouring quarters?

- **Comparison of transcriptome of control quarters from 24 hours and 72 hours *S. aureus* infection.**
- **Analysis of microarray data identified 0 genes exhibiting differential expression (FDR ≤ 0.05, fold difference ≥ 2).**
- **Less stringent analysis (P ≤ 0.01, fold difference ≥ 2) identified:**
  - **115 clones exhibit differential expression**
    - **76 annotated**
    - **73 genes**
- **75 clones (53 genes) 24 hours > 72 hours**
- **40 clones (20 genes) 72 hours > 24 hours**

DAVID analysis of *S. aureus* response

GO Biological Process term enrichment:

- **Response to stress**
- **Cell proliferation**
- **Cell development**
- **Immune response**
- **Defence response**

Genes expressed at higher levels in 24 hr infection control vs 72 hr control

- **Lactotransferrin (bactericidal peptides, bind iron preventing up-take by bacteria & binds LPS)**
- **Superoxide dismutase 2 (antioxidant)**
- **Damage associated molecular pattern (DAMP) molecules**
  - **S100 calcium binding protein A8 (calgranulin A)**
  - **S100 calcium binding protein A12 (calgranulin C) (produced by granulocytes)**
- **Conclusion: *S. aureus* infection of neighbouring quarters also triggers a response in the control quarters, which has waned by 72 hours post infection.**

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