Farm animal proteomics- from a Systems Biology perspective

Emøke Bendixen

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Hinxton
Faculty of Agricultural Science

Off-Campus RC
- 600 colleagues
- >2000 animals

Institutes:
- Genetics,
- Animal Health and Biosciences
- Food Science
Proteomics in farm animals
Biomarkers for:

Solving the problems of farm industry

- Health
- Growth
- Fertility
- Milk (yield and quality)
- Meat (yield and quality)

Model organisms for human biology
Studying biological variation - the key to characterising biological systems

• Biologic variation through natural selection
Biological variation in Farm animals - originates from **selective breeding**

Breed: Jersey
Milk Production (50 L/day)
**Biology:** complex genetic influence (remains to be characterised)

Breed: Belgian Blue
Meat production (5Kg muscle/day)
**Biology:** Myostatin gene mutation
Biological variation in Farm animals - originates from selective breeding

Breed: Danish Landrace
Meat production
Lean growth

Breed: Hungarian Mangalica
Fat production
Obese growth

Model animals

Farm animals are very special sets of biological samples, that allow studies of extreme physiology.
Pig as a model organisms for human disorders:

- Genome homology
- Metabolism (omnivore)
- Brain anatomy
- Body size
- Organ size
- Gut physiology

Pig production…25 mio/year (DK)
- Documentation
- Book keeping
- Lines of breeds…variation

Genetic variation intensly studied and well documented
Our pig genome resources

**Trait-genome mapping**
- Genetic variation in a 12,000 animal family (12 boars)
- >20 tissues collected
- Growth traits linked to genetic variation (SNPs and QTLs)
  - Metabolism
  - Obesity-fat deposition
  - Lean growth

**Genome**
- Complete, but still not fully annotated
- 4 genomes completed

**Transcriptome**
- cDNA arrays (27K)
- Oligo-arrays (24 K)
- mRNA quant (454-reads)
- Tag based (Sage-Solexa)
- Small RNAs (Solexa-454)

**SNPs**
- 50 K arrays

**Transgens**
- Parkinson (a-synuclein)
- ALS (SOD1-G93R)

**Source of animal models**
Todays talk:

Proteome studies related to gut health and metabolism

Proteome studies related to mammary gland health
Gut health in Industrial pig production

- 25 million pigs/year (Denmark)
- Neonatal mortality 5-10%
- 90% deaths → 1st week
- Mostly related to "gut problems"
- An intibiotics-issue (the resistence problem)

- Ethical and economical problem !!!!!
Host bacteria interactions in gut

Functions of gut epithelium

- Nutrient uptake
- Frontier
- Defense
- Knowing friend from enemy

Gut cell plasticity

Controlled by genes and environment!
Phenotypes in germ-free animals

**Gut morphology and function**
Villi are longer and thinner
Capillary networks are reduced

**Metabolism**
Extract less energy from diet
Lipid metabolism altered

**Immunology**
Reduced secretion of IgA
Smaller Peyers patches
Reduced number of M-cells
Decreased production of antimicrobial proteins
Impaired regulatory T-cell development
Gnotobiotic pig model

Does different gut bacteria have different impacts on gut tissue development?

12 germ-free piglets

4 x Maintained germ-free

4 x Lactobacillus fermentum

4 x Escherichia coli
Gnotobiotic pig model

Does different gut bacteria have different impacts on gut tissue development?

- Maintained germ-free
- Lactobacillus fermentum
- Escherichia coli
- Complex microbiota

Decreasing villus lengths

iTRAQ-labeling

Peptides from 4 different samples are labelled with unique mass tags

Reference - 114
GF - 115
LF - 116
EC - 117
Regulated proteins

Contrasts:
- LF/GF – least regulated proteins
- EC/GF – most regulated proteins

61 significantly regulated proteins

AGREEMENT WITH MORPHOLOGY OBSERVATIONS
Colonization stimulates the formation of blood vessels in villi


Colonization stimulates the formation of blood vessels in villi

Gnotobiotic pig - results

Both *L. fermentum* and *E. coli*:

- Affected lipid metabolism
- Stimulated the formation of new blood vessels

*E. Coli* selectively stimulated:

- enterocyte cell migration (actin re-modeling)
- cell proliferation

Conclusion: Yes, different bacteria does have different impacts on the tissue development

(Danielsen et al, 2007, Journal of Proteome Research)
Gut microbiota and obesity

Mice that inherit obese-biota gain more weight!!!

Host-bacterial interaction

- 10 x bacteria than human cells
- 500-1500 bacterial species

Ultra-complex functional genomics!
Infectobesity: - a co-culture screening approach

Co-cultures:
- Bacteria isolates (120)
- Intestinal epithelia cells
- Co-incubation/culture

Proteomics:
- Protein extraction
- LC-MS/MS
- iTRAQ technology

Pathway analysis:
- Fat metabolism
- Growth
- Adhesion
- Metabolic profiles
- Immunity
Human fetal small intestinal cells (FHs74Int) was co-incubated with *Bacteroides fragilis* and *Lactobacillum acidophilus*.

Results of proteomics from 2 hour incubation:

- Glycolysis ↓
- Detoxification ↑↓
- Lipid metabolism ↑
- Ribosomal proteins ↓
- Cytoskeleton proteins ↑
co-cultures with Ecoli K-12

- Cell culture inserts

FHS74Int: 0, 2, 8 and 24h

enzymes involved in CH metabolism
- Glyceraldehyde-3-phosphatase ↓
- Fructose-biphosphate aldolase A ↓
- Triosephosphate isomerase ↓
- Alde-keto reductase family 1 member B10 ↓

Other proteins
- ↓ proteins involved in energy metabolism
- ↓ inflammatory and pro-apoptotic proteins
- ↑ proteins involved in cell growth

Next step: SRM-based methods for screening selected metabolism markers
Mastitis in dairy cows

- Inflammation of the udder and mammary gland
- Several pathogens can cause mastitis
- The major problem in dairy cattle industry
- Affects 10% of lactating cows
- Major genetic influence on host response (QTLs)
- A major welfare and economic issue
Diagnosis of mastitis

Current methods:

The aim:

early biomarkers

On-line detection in milking robots

proteomics
iTRAQ-analyses of milk proteins after LPS challenge

MILK

Reference – time= -3

Time =0

Time=4

Time=7

Protein extraction

iTRAQ technology

LC-MS/MS
Host response to LPS, detected in milk from 3 individual cows
(Danielsen et al., Proteomics, 2010)

Acute phase response

Calgranulin B
Regulation of Apo (A) after LPS challenge
From shot-gun to targeted proteomics

Shot-gun proteomics

- Discovery-based
- Relative quant
- High abundant proteins
- ~100 most abundant milk proteins detected

Targeted proteomics

- Hypothesis-based
- Absolute quant
- Low abundant proteins

Detection range: $50 - 10^6$ copies/cell

(Picotti et al., 2009, Cell 138, 795-806)
Building Peptide Atlas of pig and cattle

- Collaboration with Institute for Systems Biology, Seattle
- (Eric Deutch, Therry Farrah & co)
- Bovine atlas;
- Milk, mammary gland, cultured cells, Immune cells,
- >2000 proteins
- > 20 000 peptides
- Porcine Atlas:
- > 20 different tissues
- > 7000 proteins
- > 50 000 peptides
Proteotypic peptides

<table>
<thead>
<tr>
<th>Peptide Accession</th>
<th>Pre AA</th>
<th>Peptide Sequence</th>
<th>Fol AA</th>
<th>Suitability Score</th>
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<th>EOS</th>
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Protein Coverage = 25% (31.4% of likely observable sequence)

View peptides and related proteins as a network with Cytoscape

[View peptides and related proteins as a network with Cytoscape]
Selected reaction monitoring (SRM)

Identification

Peptides → Electrospray ionization → MS precursor ion selection → Fragmentation → Fragment ion selection → Transition

Quantification

Spike "heavy" peptides → Sample "light" peptides → Ratio "light" peptide to "heavy" peptide
Transitions for 20 target-proteins have been optimised.

### High-abundant proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>Number of peptides detected</th>
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<tbody>
<tr>
<td>Lactoferrin</td>
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</tr>
<tr>
<td>Alpha-1-antitrypsin</td>
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</tr>
<tr>
<td>Alpha-2-macroglobulin</td>
<td>4 / 4</td>
</tr>
<tr>
<td>LGALS1</td>
<td>3 / 4</td>
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<tr>
<td>LGALS3</td>
<td>3 / 4</td>
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<tr>
<td>SAA3</td>
<td>3 / 3</td>
</tr>
<tr>
<td>S100A12 - Calgranulin C</td>
<td>4 / 4</td>
</tr>
<tr>
<td>Calgranulin B</td>
<td>4 / 4</td>
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<tr>
<td>Cathepsin C</td>
<td>3 / 4</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>4 / 4</td>
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</table>

### Low-abundant proteins

<table>
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<td>Vanin 1</td>
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QconCat – 40 peptides

Synthetic peptides → Q-TRAP → [Graph showing data]
Quantitation by heavy QconCat peptide spiking and SRM.

Sample: "light" peptides

Spike: heavy QconCat – 40 peptides

Co-digest

Ratio "light peptide to "heavy" peptide

Absolute quant (fmol/ug)
Milk samples spiked with (H)QconCat peptides

LPS challenge

<table>
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<tr>
<td>VAN1</td>
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<td></td>
</tr>
</tbody>
</table>
LPS challenge

A2M

Concentration, fmol/μg

HPG

Concentration, fmol/μg

LPS challenge
Relative versus Absolute quant data

Calgranulin B

iTRAQ

Spiked with 5 fmol(H)QconCat peptides

CALGB
“We must measure what can be measured, and make measurable what cannot be measured.”

Galileo Galilei 1610
Farm animals provide important biological variation for systems biology

Solving the problems of farm industry

Model organisms for human biology
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