Microbial feed additives

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Projects

The C-ex project (2003-2005) — CRAFT / FP5

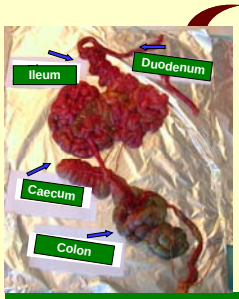
The ProPig project (2005-2008) — FFG

The GUT-Function project (2009-2012) — FFG

❖ Development of multi-strain feed additives for poultry/swine meeting the requirements for registration within the EU

Building the BRIDGE

The Gut Microbiota



Gastrointestinal tract

Selective enrichment using various media & conditions

1) aerobic
2) facultative anaerobic
3) strict anaerobic

Result: pool of ~ 100 diverse isolates

❖ The gut contains at least 400 different bacterial species, a total of ~ 1000 microbes.

❖ Gut bacteria are by nature in constant competition with other microbial species.

Strain selection ...

- physiological
  adapted to the GI-tract
  tolerant to acidic condition
- functional
  e.g. inhibition of pathogens
  (barrier function)
- technological
  cultivable to high cell density
  robust
- safe
  no virulence genes
  no antibiotic resistance genes

❖ What happens *in vivo* after feeding the microbes?

❖ Do our feed supplements affect the composition of the gut microbiota or the hosts' immune system?

❖ What happens during intestinal disorders (*e.g.* infection) ?

❖ Can we follow our bacteria along their GI tract journey ?

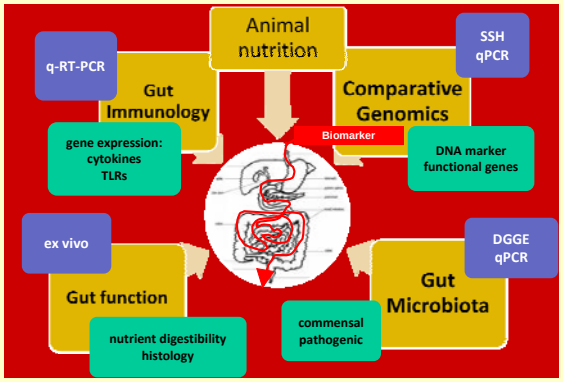
The Basic Facts

The gut is the largest interface between the feed and the animals' microflora and immune system.

Microbial feed additives might affect this interface, especially at times of need (*e.g.* stress upon weaning, infections, post-hatching).

Feeding trials have shown promising effects, however, "only" on the level of "easy-to-measure" performance (body weight, feed conversion, ..).

Motivation



The ultimate goal is to create a link between *in vitro* data and animal performance & health.

We approach these questions by using ..

❖ quantitative (q) RT-PCR to study the up- and down-regulation of immune marker genes (*e.g.* cytokines, TLR) in host tissues (*e.g.* spleen)

❖ qualitative & quantitative PCR approaches (DGGE, qPCR) to measure shifts of bacterial populations (commensal, pathogens) in the gut habitat

❖ *ex vivo* techniques to study gut morphological changes

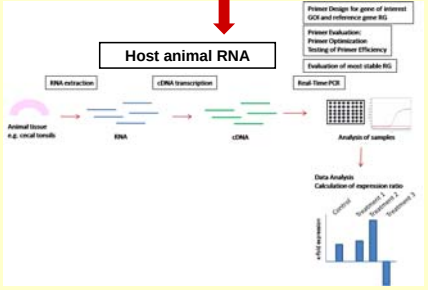
❖ biomarkers for specifically tracking of probiotics within the complex GI tract ecosystem

Assessing the effects of nutrition on…

1) Gut immunology

Immunological tissue
- cecal tonsils
- peyer's patch
- spleen

Host animal RNA

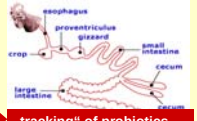


Total RNA is extracted from tissues and reverse transcribed to cDNA. Real time PCR is performed using primers specific for the targeted genes. Housekeeping genes are included as quality control for the test sample cDNA.

2) Gut microbiota

GI tract sample
- lumen contents
- mucosa
- faeces

Quantification of gut bacteria



„tracking" of probiotics

Probiotic strain — Biomarker

Bacterial DNA — quantitative — Real time PCR

- commensal
- pathogens

-16S rDNA
- IGS DNA

GENOMICS
- Probiotic strain

Finding unique genomic sequences in the strain of interest for specific detection & quantification by real time PCR

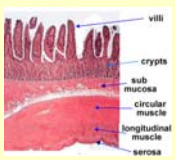
- Suppression subtractive hybridisation (SSH)

PCR — qualitative — cloning — primer design

DGGE — sequencing — phylogenetic analysis

identification of bands

3) Gut architecture (histology)



Ref.: 1) Klose *et al.* Mol. Nutr. Food Res. 2006, 50, 563– 71 2) Klose *et al.* J Appl Microb 2009, 108, 1271- 80 3) Klose *et al.* 2010 Vet Microbiol (in press)