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Translational pig model for microbiota management and its effects on intestinal health

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Objective

To demonstrate the relevance of pig models for human intervention strategies we performed an intervention study in piglets, and assessed responses to human probiotics *in vivo* and *in vitro*

Methods *in vivo* study

The effect of two probiotics, *Lactobacillus plantarum* and *Lactabacillus caseii* were compared to placebo treatment. Three groups of six 6 week old piglets were treated daily with probiotics for a period of 8 consecutive days, after which they were killed. At necropsy, different locations of the GI tract were sampled for evaluation of epithelial integrity, microbiota composition, and transcriptional host responses.

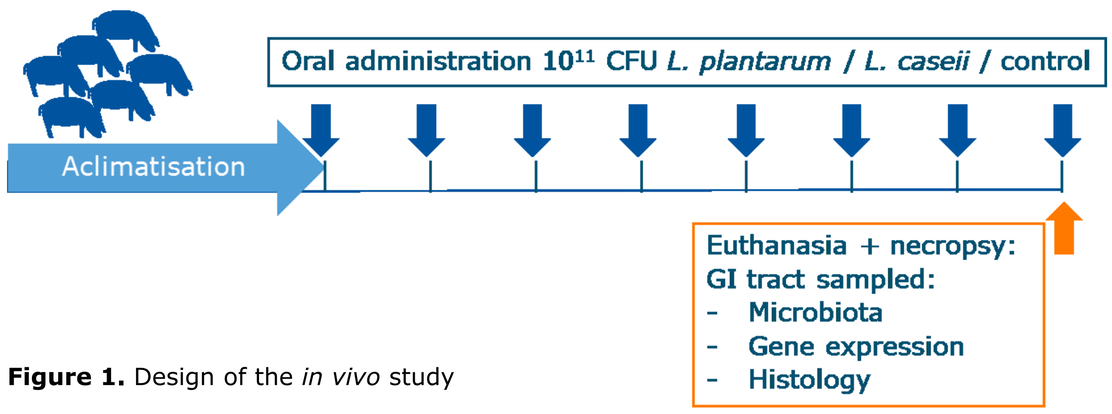
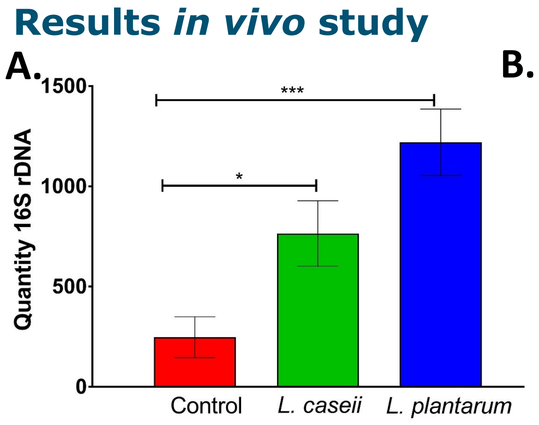
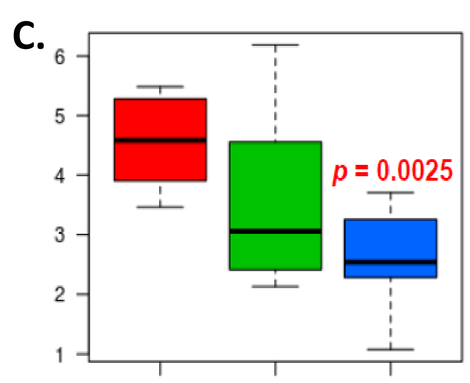


Figure 1. Design of the *in vivo* study

Results *in vivo* study





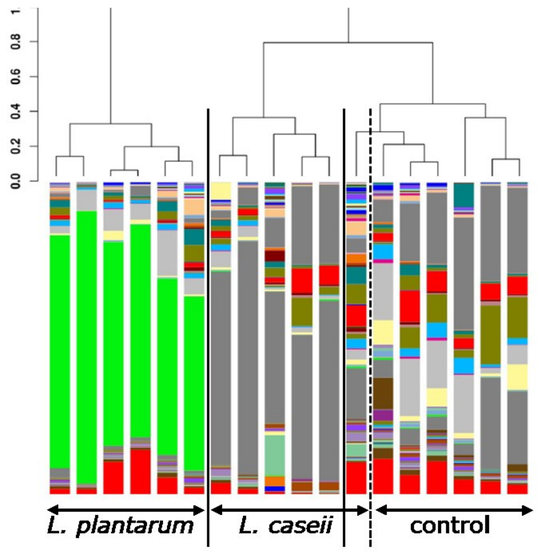


Figure 2A. 16S rDNA microbiota quantity comparison between treatments. *** indicates p<0.001, * indicates p<0.05
Figure 2B: Hierarchical clustering of microbiota of jejunum samples
Figure 2C: Shannon diversity (accounts for both abundance and evenness of species) comparison between treatments in Jejunum

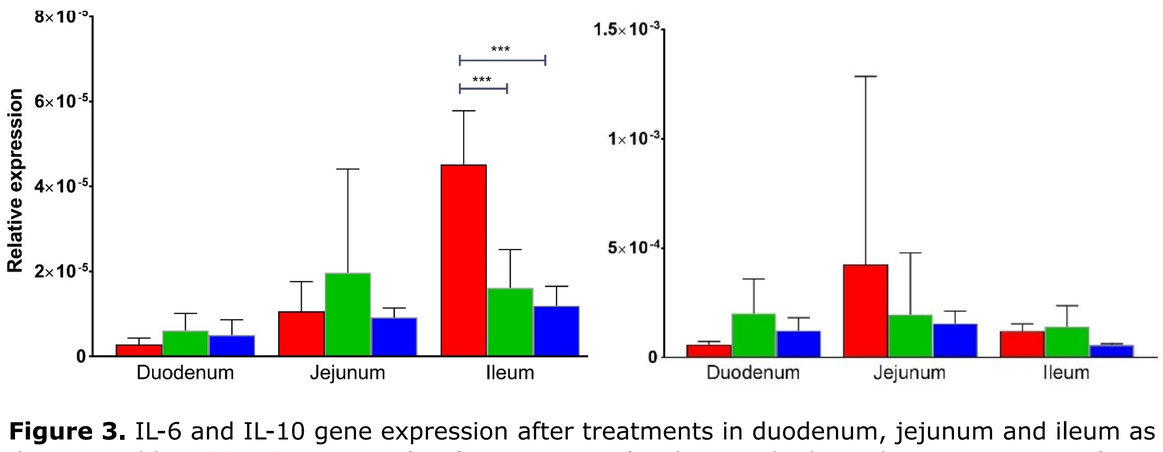


Figure 3. IL-6 and IL-10 gene expression after treatments in duodenum, jejunum and ileum as determined by qPCR. Expression levels were normalised using the housekeeping gene ppi (n = 6). Error bars indicate SEM, *** p < 0.05.

Results *in vivo* study

Administration of probiotics to pigs increased microbiota quantity (figure 2A), and induced changes in microbiota composition (figure 2B) and diversity (figure 2C). Both probiotic isolates induced these differences, but the treatment groups could be discriminated based on their microbiome, indicating that both strains induced differential effects.

A decrease in *il6* gene expression, but not in other immune genes, was observed in ileum, but no other intestinal part (figure 3).

Methods *in vitro* study

To evaluate whether probiotics can affect disease outcome, an *in vitro* model using porcine intestinal cells (IPEC) was used. IPEC cells were treated with *L. plantarum*, and subsequently challenged with an *E. coli* strain (ETEC) (figure 4).

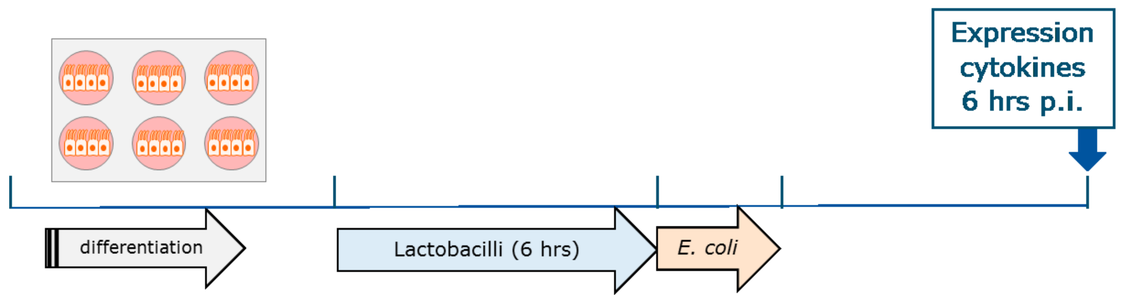


Figure 4. Design of the *in vitro* study

Results *in vitro* study

Probiotics affected the immune response to E. coli, reducing IL-6 and IL-10 expression compared to IPEC cells only challenged with *E. coli*.

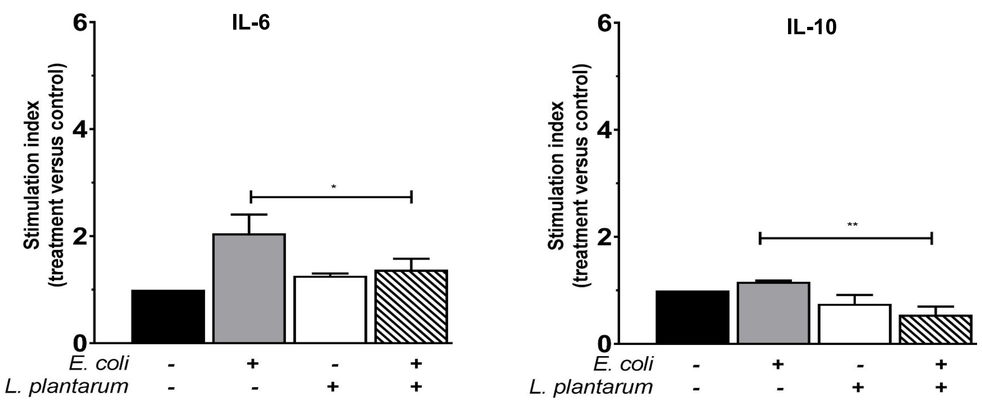


Figure 5. IL-6 and IL-10 secretion in IPEC cells 6 hours post treatment (n = 6). Error bars indicate SEM, ** p < 0.01, * p<0.05.

Conclusions

- Administration of probiotics to pigs induce changes in microbiota composition and immune gene expression.
- *In vitro* results indicate that probiotics affect the immune responses induced by *E. coli*. These responses are comparable to probiotic effects of dampening the proinflammatory immune response in humans.

Pigs are very suitable as translational models to study human intestinal health due to their comparable anatomy, intestinal microbiota, nutritional requirements, and inflammatory responses. New pig studies will be performed where the ameliorating effect of probiotics will be studied *in vivo*. In addition, the immune responses will be studied more extensively by including flow cytometry analyses of gut and serum immune cells, and more comprehensive cytokine profile analyses.

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