
HOST RESPONSE AND MICROBIOTA COMPOSITION AFTER LACTOBACILLUS ADMINISTRATION: PIG AS MODEL FOR HUMAN

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Effect of probiotics on intestinal gene expression and microbiota composition

■ Pigs good model for human GIT

- Comparable anatomy
- Omnivores
- Comparable lifespan

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A pig model of the human gastrointestinal tract

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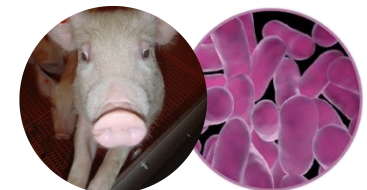
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Keywords: pig model, intestinal microbiome, 16S rRNA amplicon sequencing, principal coordinate analysis, microbial diversity

■ Animal model to study probiotics increases possibilities

- More standardized conditions
- Sampling along GIT
- Additional challenges possible (viral or bacterial pathogen / stress /)

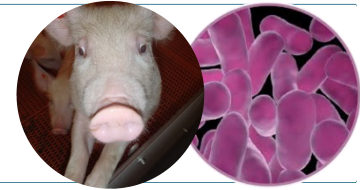


AIM

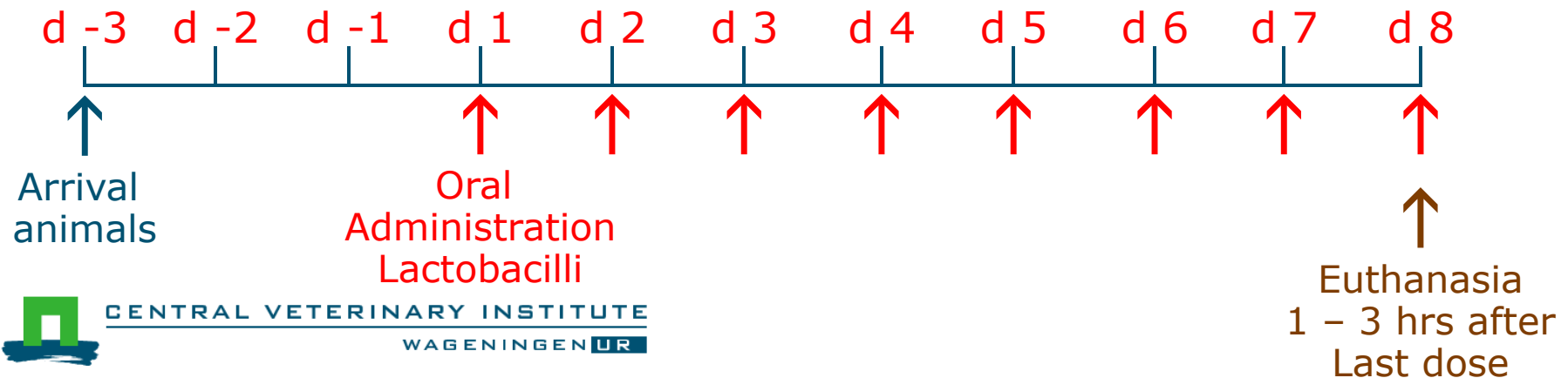
- The potential of porcine models for probiotic studies.
- This study evaluates the porcine responses to probiotics for which the molecular responses were previously determined *in vivo* in humans
- Intestinal gene expression and microbiota were determined after oral administration of *Lactobacillus*



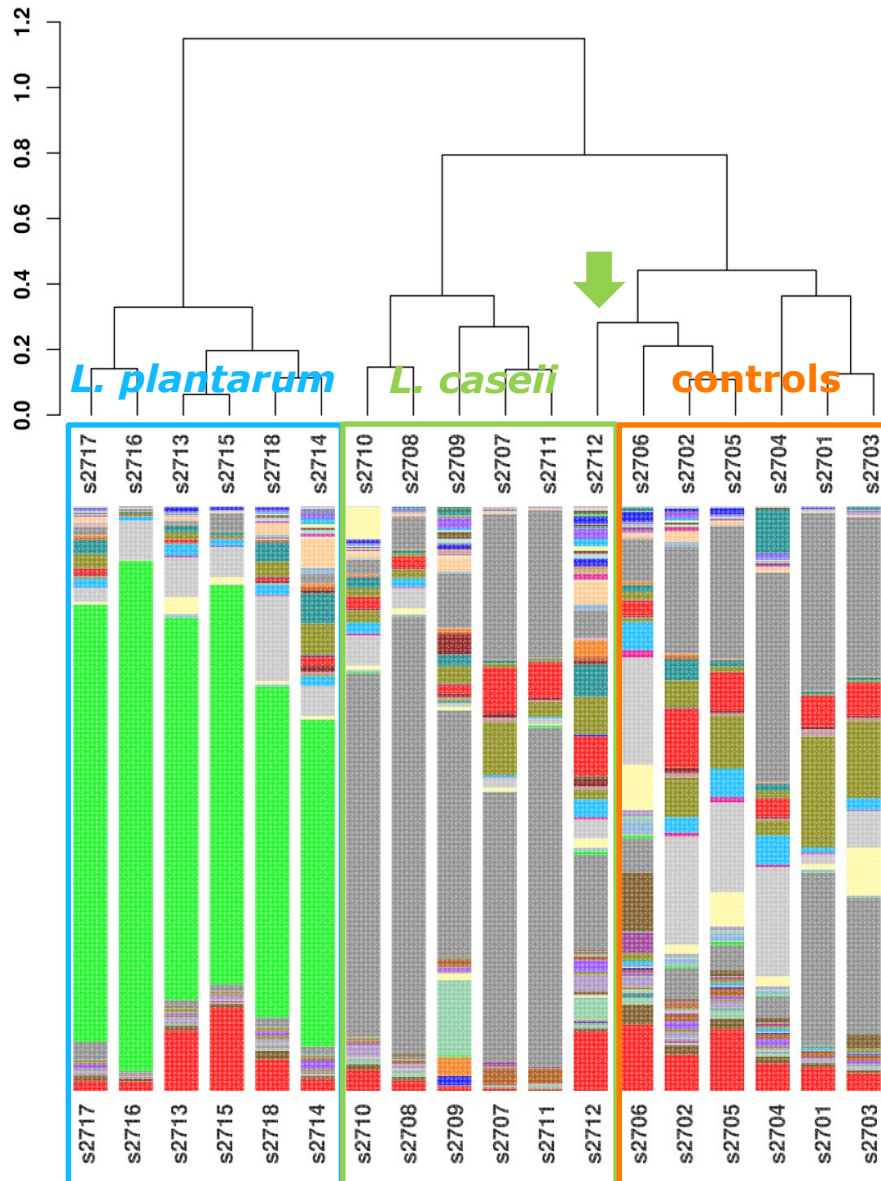
Experimental set-up



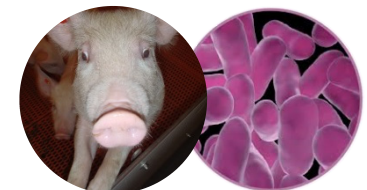
- 3 groups of 6 6-week old piglets (♀):
 - *Lactobacillus caseii* (glycerol stock in sport drink)
 - *Lactobacillus plantarum* (glycerol stock in sport drink)
 - Control (glycerol in sport drink)
- Daily administration orally ($\sim 4 \cdot 10^{11}$ CFU)
 - Daily consumption advised for human probiotics
 - Based on thesis Gabriele Gross: *L. plantarum* does not colonize in piglet GIT



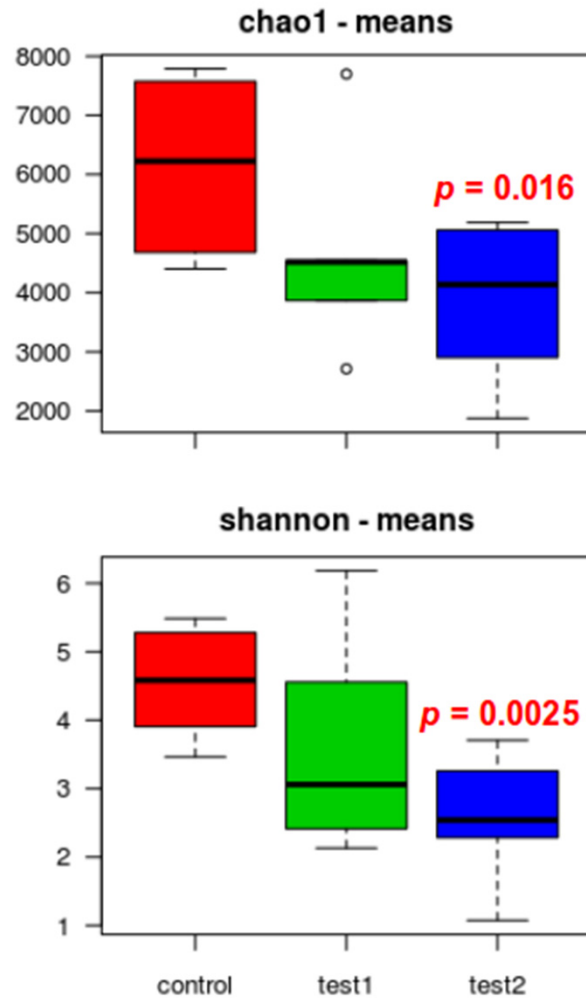
Clustering based on microbiota profiles



- Animals cluster according to treatment
- *L. plantarum* treatment yields most distinct group
- One *L. caseii* animal clusters with control animals (S2712)



Diversity of microbiota

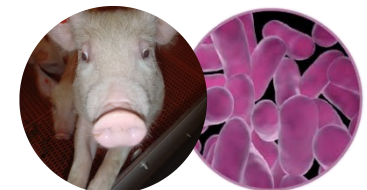


- Alpha-diversity of lactobacillus treated groups reduced
- *L. plantarum* treatment significant reduction in diversity
- *L. caseii* treatment reduced diversity, but not significant

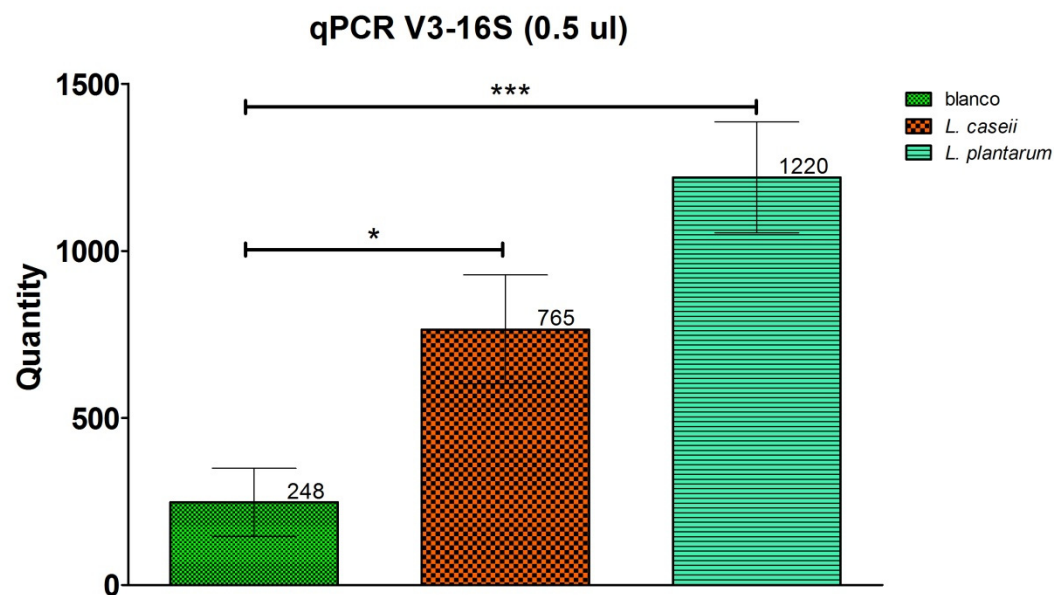
L. plantarum

L. caseii

controls

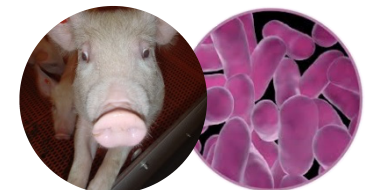


Absolute amount microbiota

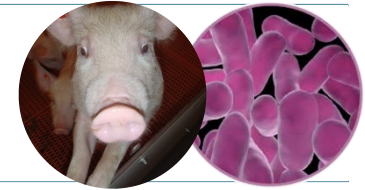


- Increase in microbiota in lactobacillus treated groups
- *L. caseii* 3 times more than control
- *L. plantarum* 5 times more than control

Differences between treatment groups are smaller than suggested.



Taxon – treatment association



- *Lactobacillaceae* are increased in both *L. plantarum* and *L. caseii* treated piglets ($p = 0.0079$)
- *Lactobacillaceae* in *L. caseii* group are predominantly *L. caseii*, same for *L. plantarum*
- OTU association shows decreased diversity among *Lactobacillaceae*

	control	<i>L. caseii</i>	<i>L. plantarum</i>
<i>Lactobacillaceae</i> species	65	37	22

- Increased *Lactobacillaceae* at dispense of other taxons:

Genus	control	<i>L. caseii</i>	<i>L. plantarum</i>
<i>Peptostreptococcaceae</i>	23.7	12.1	1.2
<i>Streptococcaceae</i>	13.1	2.5	6.8
<i>Leuconostocaceae</i>	1.9	0.26	0.26
<i>Clostridiaceae</i>	5.5	4.3	0.6
<i>Gemella</i>	0.4	0.04	0.04



Gene expression intestine

- LIMMA (Linear Models for Microarray Data)
- $P_{adj} < 0.1$ and $\logFC > |1.3|$

Tissue	Comparison	Probes		Annotated Genes	
		down	up	down	up
Duodenum	<i>L. caseii</i> vs. Control	0	0	0	0
	<i>L. plantarum</i> vs. Control	70	5	25	3
Ileum	<i>L. caseii</i> vs. Control	10	14	8	2
	<i>L. Plantarum</i> vs. Control	43	154	38	45



Functional Analysis (2)

L. Plantarum vs Control (Ileum)

Down-regulated genes *L. plantarum* vs control (ileum)

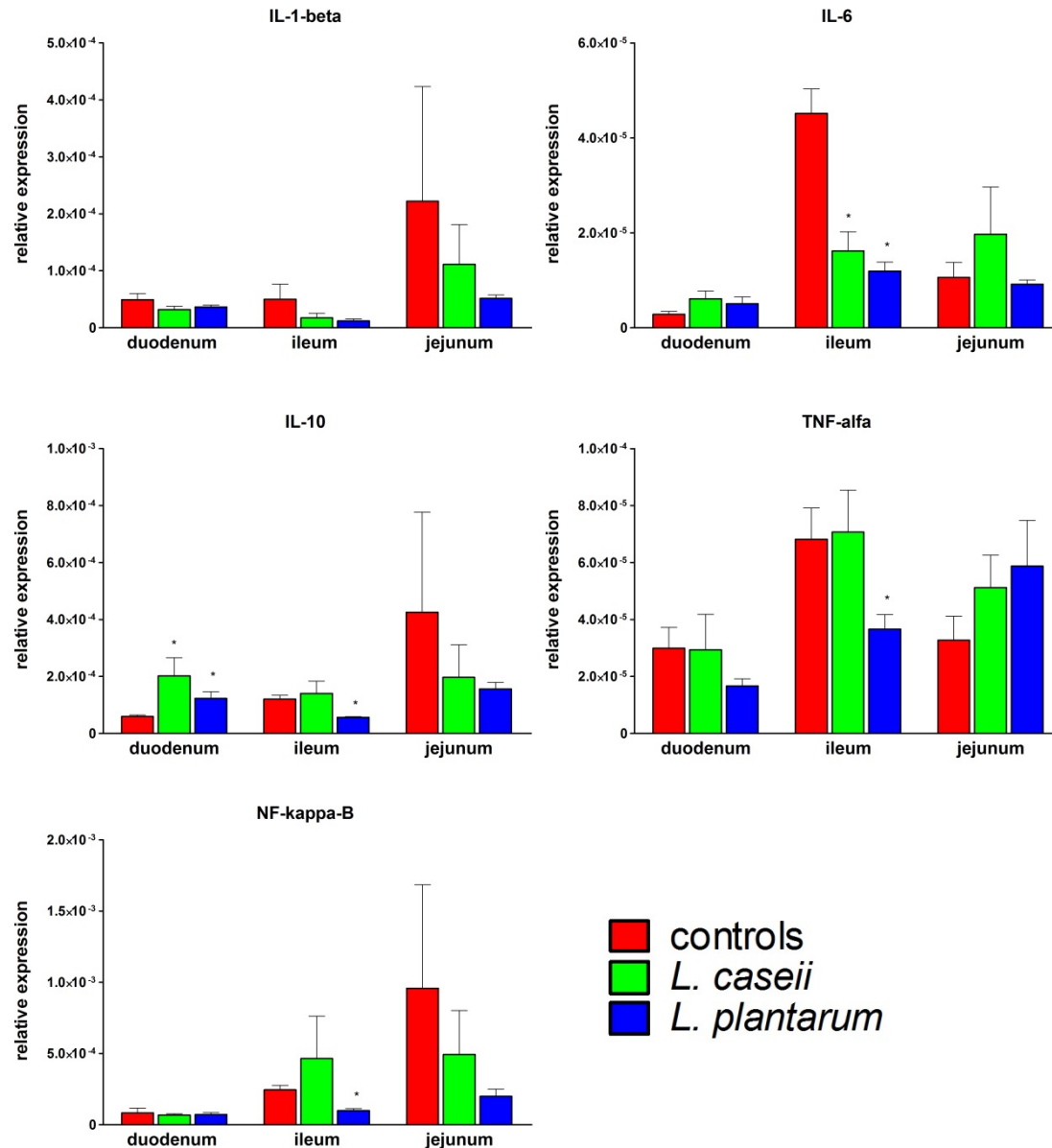
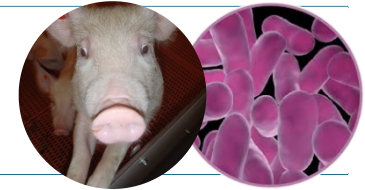
Term	Overlap	Combined Score	Genes
immune system process (GO:0002376)	6_552	6.7	HLA-DRA;CD209;TREM1;SAMHD1;LST1;FCAR
immune response (GO:0006955)	5_421	5.9	HLA-DRA;TREM1;SAMHD1;LST1;FCAR
regulation of innate immune response (GO:0045088)	2_26	4.7	SAMHD1;SERPING1
cell adhesion (GO:0007155)	5_319	4.7	POSTN;CCR3;CD209;SELL;ZYG
regulation of immune response (GO:0050776)	2_96	3.2	SERPING1;SAMHD1
hormone metabolic process (GO:0042445)	2_50	2.7	HSD11B2;ACE
lipid biosynthetic process (GO:0008610)	2_121	2.7	ACSS2;HSD11B2
HSA04510 FOCAL ADHESION	2_200	2.6	COL6A2;ZYG
HSA04514 CELL ADHESION MOLECULES	2_134	2.3	HLA-DRA;SELL
anatomical structure development (GO:0048856)	4_639	2.2	POSTN;ACE;BMP4;LST1

Up-regulated genes *L. plantarum* vs control (ileum)

Term	Overlap	Combined Score	Genes
establishment or maintenance of chromatin architecture (GO:0006325)	3_166	4.8	HIST1H2BN;EP400;WHSC1L1
chromosome organization (GO:0051276)	3_225	4.6	HIST1H2BN;WHSC1L1;EP400
organelle organization (GO:0006996)	3_553	3.0	HIST1H2BN;WHSC1L1;EP400
chromatin modification (GO:0016568)	2_125	2.9	EP400;WHSC1L1
regulation of transcription from RNA polymerase II promoter (GO:0006357)	2_334	2.7	ELL3;CHD3
protein modification process (GO:0006464)	3_736	2.7	WHSC1L1;LRP2;EP400
HSA04810 REGULATION OF ACTIN CYTOSKELETON	2_212	2.7	PFN2;LIMK2
HSA04670 LEUKOCYTE TRANSENDOTHELIAL MIGRATION	2_115	2.4	CLDN7;ARHGAP5
cellular protein metabolic process (GO:0044267)	3_963	2.2	WHSC1L1;LRP2;EP400
RNA metabolic process (GO:0016070)	2_498	2.2	SYNCRIP;ELL3



qPCR @ innate immune genes



- Differences between GIT locations

- Largest variance in jejunum
- Duodenum least responsive

- *L. caseii* & *L. plantarum* suppress IL-6 (and IL-1-beta)

- *L. plantarum* suppresses TNF-alfa as well

- IL-10 response ambiguous

- NF-kappa-B1 (p105/p50) suppressed by *L. plantarum*

Conclusion

- No gross morphological changes in epithelial integrity were observed.
- Microbiota composition of probiotic groups changed
 - the administered lactobacilli were among the most predominant species residing in the jejunum, although lactobacilli were also prominently present in the control animals.
- *L. plantarum* administration elicited transcriptional modulation of immune related pathways in ileum, which was also the most prominent response-category observed in the human study



Are probiotics beneficial for health?

- Can probiotics affect course of infection/infectious disease
- requires → animal model to measure in an appropriate window of infectious disease (subclinical-mild)
- At CVI two porcine models for respiratory or systemic infection used:
 - → mild virulent bacterial infection (*Actinobacillus pleuropneumoniae*) alone or preceded by subclinical viral infection (*Porcine Reproductive and Respiratory Syndrome Virus*)
 - Mild symptoms of animals allows assessment of effects of treatments like feed or management

Credits

Host Microbe Interactomics

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Animal Care Takers

