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Effect of the Probiotic *Enterococcus faecium* NCIMB10415 on Cell Numbers of Total *Enterococcus* spp., *E. faecium* and *E. faecalis* in the Intestine of Piglets

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Abstract

Sows and their piglets were fed a diet supplemented with or without the probiotic *E. faecium* NCIMB10415 (also known as SF68). Piglets were sacrificed 14, 28, 35 and 56 days after birth and DNA from intestinal segments was extracted and purified. A real time PCR assay was used to distinguish *Enterococcus* spp. (16s rDNA based), *E. faecium* (*Efaafm* gene), *E. faecalis* (*Efaafs* gene) as well as the probiotic strain (unique plasmid sequence). Extracts of autoclaved sow feces inoculated with *E. faecium* and *E. faecalis* cultures were used to calibrate real time PCR results. The probiotic strain was detected in 14 day old suckling piglets before the piglets had access to the starter diet. In piglets of the probiotic group, probiotic *E. faecium* cell counts were always a significant proportion of total *E. faecium* cells in stomach digesta (4–20%), however only a small fraction of the total *Enterococcus* spp. cell number on day 14 and 28 in all intestinal segments (0.1–0.7%). Compared to control samples, the probiotic *E. faecium* strain significantly ($p \leq 0.05$) decreased the amount of total *Enterococcus* spp. and *E. faecalis* cells in the colon of 14 day old suckling piglets as well as in jejunum and colon samples one week after weaning. *E. faecium* cell counts were not modified on any sampling day or intestinal segment.

This study showed that the presence of probiotic *E. faecium* NCIMB10415 coincided with reduced total *E. faecalis*, but not total *E. faecium* cell numbers in the intestine of piglets. In view of unchanged cell numbers and ratios in sow feces, modifications must have taken place within the intestine of suckling piglets.

Introduction

Probiotics are increasingly used in the EU in an effort to counter the ban on antibiotics in animal nutrition. Beneficial effects have been documented (Underdahl, 1983, Maenner and Spieler, 1997, Kyriakis *et al.*, 1999, Taras *et al.*, 2006), but the modes of action remain a matter of discussion and ongoing research. *Enterococcus faecium*, *Bacillus* spp. – and yeast strains are the major commercially available probiotic products in animal nutrition in the EU. The intestinal bacterium *E. faecium* NCIMB 10415, also known as *E. faecium* SF68, is the active ingredient of some probiotic products. This strain has already been shown to invoke effects on piglet performance and on general composition of the intestinal

microbiota (Maenner and Spieler, 1997; Pollmann *et al.*, 2005; Scharek *et al.* 2005) as well as modifying the immune response of piglets (Scharek *et al.* 2005). Furthermore, an enterocin of this strain was shown to inhibit growth of *E. hirae*, *E. casseliflavus*, as well as 4 of 12 *E. faecium* strains, but only one of 10 *E. faecalis* strains (Moreno *et al.*, 2003).

A variety of porcine intestinal enterococci have been isolated (Devriese *et al.*, 1994). *E. faecalis*, *E. hirae* and *E. faecium* seem to be the major cultivable *Enterococcus* spp. in pigs (Kuhn *et al.*, 2003). However, Leser *et al.* (2002) did not find a large proportion of enterococci by 16s rDNA amplification/cloning of pig ileum and colon DNA. This may indicate that enterococci are not a major part of the total intestinal microbiota of pigs. Nevertheless, enterococci carry resistance genes against a range of antibiotics (Hasman *et al.*, 2005, Jackson *et al.*, 2005), which may be important for pig health as well as consumer safety.

This study investigated the effect of a probiotic *E. faecium* strain on total intestinal *E. faecium* and *E. faecalis* cell numbers in order to detect probiotic induced interactions between these closely related species in the intestine of piglets.

Results and discussion

Realtime PCR assays

This study used realtime PCR assays to quantify cell numbers of enterococci in the intestine of piglets. Specificity of the employed primers were verified against a range of reference strains and isolates, but no falsepositive results were observed with any culture DNA extract. Thus, all employed primers were considered to be specific for their respective target DNA. This also confirms again the specificity of the primer combinations chosen by Eaton and Gasson (2001) to identify *E. faecium* and *E. faecalis* species as well as the primer pair for the detection of *Enterococcus* spp. by Rinttilä *et al.* (2004).

Calibration was achieved by spiking known amounts of cells from two *E. faecium* and two *E. faecalis* strains or from *E. faecium* NCIMB10415, respectively, into autoclaved sow feces and subsequent nucleic acid extraction. Calibration curves for *Enterococcus* spp. (four strains) were linear from 10^3 to 10^8 cells per gram sample, corresponding to amplification thresholds (c_t) of 11.3 (± 0.5 , $n=3$) to 32.5 (± 0.9 , $n=3$). Linearity for single species extended from 10^4 to 10^8 cells per gram sample with c_t values of 15.1 (± 0.5 , $n=3$) to 35.8 (± 1.2 , $n=3$). Lower cell numbers increased the standard error, possibly due to incomplete homogenization of target cells in autoclaved feces and poor nucleic acid recovery at low cell concentrations.

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Sow feces was used as the most difficult matrix to extract DNA, because of the presence of multiple PCR inhibitors, especially of polyphenolic substances of lignins from dietary fibre, complex polysaccharides and bile salts (Wilson, 1997; Widjoatmodjo *et al.*, 1992; Monteiro *et al.*, 1997; Koonjul *et al.*, 1999). Furthermore, the use of the same matrix and extraction method for calibration and intestinal samples ruled out systematic errors due to different extraction efficiencies.

The use of DNA extracts from spiked matrices is superior to DNA extracts of cultures, because it incorporates the lower PCR amplification rate due to enzyme inhibition in complex sample extracts. Overestimation of enterococci due to the use of culture extracted genomic DNA for calibration has been reported by He and Jiang, 2005.

The calibration sample with 4×10^8 cells/g total enterococci (1×10^8 cells/g for each of the four single strains) was serially diluted and amplified in order to determine PCR amplification efficiencies of the assay. The supplied stratagene computer software calculated the PCR amplification efficiency to be 89.2% (± 5.7 , $n=3$) for the *Enterococcus* spp., 104.2% (± 2.4 , $n=3$) for *E. faecium*, 97.2% (± 3.9 , $n=3$) for *E. faecalis* and 102.7% (± 6.4 , $n=3$) for *E. faecium* NCIMB10415. Dilution of random samples ($n=9$ of 120) in water and subsequent PCR showed slight PCR inhibition, as PCR amplification efficiency was below 100% for all tested samples (84.2%, ± 3.7 , $n=9$). No differences of PCR amplification efficiency between intestinal segments were noted (data not shown).

Removal of PCR inhibitors from DNA extracts is a crucial step for quantitative PCR assays, because detection sensitivity will be drastically reduced in crude DNA solutions (Widjoatmodjo *et al.*, 1992; Monteiro *et al.*, 1997; Koonjul *et al.*, 1999). This may not be as important in hind gut samples with large amounts of bacterial DNA, where inhibitors may be diluted and still yield sufficient amounts of DNA for amplification. However, the small intestine harbours far less bacteria and thus PCR efficiency is crucial.

PCR results from diluted calibration and intestinal samples showed some PCR inhibition, but to a similar extent. The detection limit of 4×10^3 cells/g sample (total enterococci) and 1×10^4 cells/g sample (single strains) was sufficient to detect enterococci in most sample extracts. However, even after purification, PCR efficiency did not reach the maximum amplification rate. Thus, PCR inhibitors were still present and only removed by dilution. In conclusion, it is highly recommended for quantitative realtime PCR studies to examine DNA extracts by dilution and to calculate PCR amplification rates.

Cell numbers of *E. faecium* NCIMB10415 in sow feces and piglet intestine

Cell numbers of *E. faecium* NCIMB10415 in sow feces 10 days ante partum were 5.2×10^5 /g sample ($\pm 2.7 \times 10^5$, $n=5$). Colony hybridization of the same samples showed lower colony counts (1.8×10^5 colony forming units/g wet weight, Macha *et al.*, 2004). As Macha *et al.*, 2004 already had observed, the piglet intestine was colonized by the probiotic strain on and prior to day 14, however cell numbers were low with the exception of stomach contents

(Fig. 1). Higher cell numbers were found on day 56; however the probiotic strain still represented only a minor fraction of total enterococci. Nevertheless, probiotic *E. faecium* cell counts were always a significant proportion of total *E. faecium* cells in stomach digesta (4 – 20%), however only a small fraction of the total *Enterococcus* spp. cell numbers on day 14 and 28 in each intestinal segment (0.1 – 0.7%).

Results of this study on *E. faecium* NCIMB10415 show all stomach and colon sample extracts to be positive for the specific plasmid DNA sequence. In comparison, the colony hybridization results (Macha *et al.*, 2004) showed that on day 14 only one of five animals was positive for the probiotic strain in the stomach, but four of five animals showed high colony counts in the ileum and colon, whereas in the jejunum only one sample extract was positive with the Realtime – PCR assay. All fecal samples were positive in the probiotic treated group using colony hybridization as well as by realtime PCR. This indicates different detection limits for the probiotic strain in the comparison of the culture-dependent and the culture-independent techniques. This may be partially due to a reduced viability of the probiotic strain in stomach samples of very young animals, but also with low DNA – extraction efficiencies for jejunal contents.

Quantification of *Enterococcus* spp. cell numbers in feces of mother sows and in the intestine of piglets

The presence of the probiotic *E. faecium* strain in the sow diet did not modify fecal cell numbers of these species in sow feces. No significant differences were observed for total *Enterococcus* spp., *E. faecium* or *E. faecalis* cell numbers in mother sow feces (Table 1). An average of 3.3×10^7 and 5.2×10^7 total *Enterococcus* spp. cells per gram sample was recorded in control and trial group, respectively.

Cell numbers of total enterococci in piglets varied from 8.2×10^4 to 4.3×10^8 cells/g samples. Cell counts in the colon were in accordance to colony forming units on Slanetz-Bartley agar as reported for fecal samples of the same feeding trial (Macha *et al.*, 2004). Very similar cell counts were noted for *E. faecium* and *E. faecalis* cell numbers in both groups.

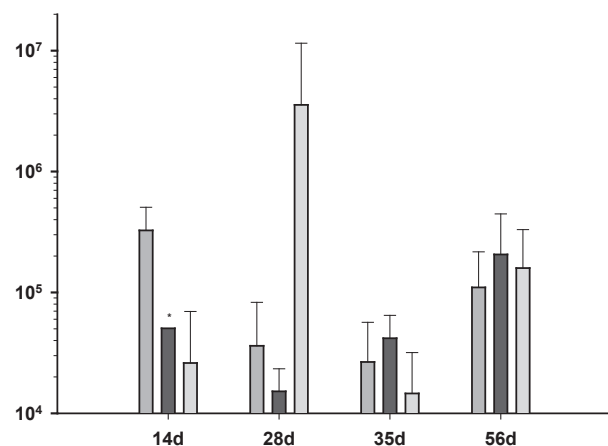


Figure 1 Cell numbers of *E. faecium* NCIMB 10415 in the intestine of piglets. ■ = Stomach, ■ = Jejunum, ■ = Colon; * = single value.

Table 1 Total *Enterococcus* spp., *E. faecium* and *E. faecalis* mean cell numbers per g wet weight (\pm SD) in the intestine of piglets and feces of mother sows as determined by realtime PCR assays (n=5 per trial group)

Age [d]	Segment	<i>Enterococcus</i> spp.			<i>E. faecium</i>			<i>E. faecalis</i>			p
		Control group	Probiotic group	p	Control group	Probiotic group	p	Control group	Probiotic group	p	
10 d ante partum	Sow faeces	3.3 × 10 ⁷ (2.3 × 10 ⁷)	5.2 × 10 ⁷ (4.0 × 10 ⁷)	0.386	9.5 × 10 ⁶ (3.5 × 10 ⁶)	1.7 × 10 ⁷ (9.7 × 10 ⁶)	0.140	1.0 × 10 ⁷ (9.8 × 10 ⁶)	1.2 × 10 ⁷ (1.2 × 10 ⁷)	0.737	
	Stomach	3.0 × 10 ⁷ (3.1 × 10 ⁷)	1.1 × 10 ⁷ (7.3 × 10 ⁶)	0.214	3.6 × 10 ⁶ (3.9 × 10 ⁵)	3.8 × 10 ⁶ (2.2 × 10 ⁶)	0.917	1.7 × 10 ⁷ (1.5 × 10 ⁷)	2.2 × 10 ⁶ (2.2 × 10 ⁶)	0.063	
	Jejunum	1.4 × 10 ⁷ (1.6 × 10 ⁷)	9.4 × 10 ⁶ (8.4 × 10 ⁶)	0.603	1.2 × 10 ⁶ (1.4 × 10 ⁶)	4.8 × 10 ⁶ (3.5 × 10 ⁶)	0.146	6.9 × 10 ⁶ (6.8 × 10 ⁶)	1.1 × 10 ⁶ (9.4 × 10 ⁵)	0.095	
	Colon	3.2 × 10 ⁷ (1.8 × 10 ⁷)	5.2 × 10 ⁶ (3.9 × 10 ⁶)	0.012	3.1 × 10 ⁶ (2.0 × 10 ⁶)	3.1 × 10 ⁶ (2.8 × 10 ⁶)	0.957	1.7 × 10 ⁷ (1.3 × 10 ⁷)	1.2 × 10 ⁶ (1.3 × 10 ⁶)	0.031	
28	Stomach	2.6 × 10 ⁷ (1.8 × 10 ⁷)	8.0 × 10 ⁶ (6.0 × 10 ⁶)	0.070	4.8 × 10 ⁶ (3.9 × 10 ⁵)	4.4 × 10 ⁶ (3.2 × 10 ⁶)	0.868	9.7 × 10 ⁶ (6.4 × 10 ⁶)	2.0 × 10 ⁶ (2.4 × 10 ⁶)	0.033	
	Jejunum	9.4 × 10 ⁶ (7.4 × 10 ⁶)	2.2 × 10 ⁷ (2.7 × 10 ⁷)	0.337	5.6 × 10 ⁵ (3.3 × 10 ⁵)	1.2 × 10 ⁷ (1.7 × 10 ⁷)	0.162	4.1 × 10 ⁶ (2.6 × 10 ⁶)	4.4 × 10 ⁶ (5.5 × 10 ⁶)	0.929	
	Colon	1.6 × 10 ⁸ (1.1 × 10 ⁸)	1.5 × 10 ⁸ (1.2 × 10 ⁸)	0.873	4.1 × 10 ⁷ (3.0 × 10 ⁷)	7.0 × 10 ⁷ (5.1 × 10 ⁷)	0.270	7.1 × 10 ⁷ (3.0 × 10 ⁷)	3.8 × 10 ⁷ (3.9 × 10 ⁷)	0.146	
	Stomach	3.3 × 10 ⁶ (2.3 × 10 ⁶)	2.9 × 10 ⁶ (3.0 × 10 ⁶)	0.840	8.1 × 10 ⁵ (9.1 × 10 ⁵)	7.9 × 10 ⁵ (7.9 × 10 ⁵)	0.959	1.3 × 10 ⁶ (1.2 × 10 ⁶)	7.8 × 10 ⁵ (1.3 × 10 ⁶)	0.535	
35	Jejunum	1.8 × 10 ⁷ (5.6 × 10 ⁶)	5.1 × 10 ⁶ (3.8 × 10 ⁶)	0.003	2.9 × 10 ⁶ (1.9 × 10 ⁶)	1.5 × 10 ⁶ (8.7 × 10 ⁵)	0.180	6.9 × 10 ⁶ (3.5 × 10 ⁶)	4.1 × 10 ⁵ (5.1 × 10 ⁵)	0.003	
	Colon	2.1 × 10 ⁸ (1.3 × 10 ⁸)	3.8 × 10 ⁷ (3.4 × 10 ⁷)	0.020	5.0 × 10 ⁷ (3.3 × 10 ⁷)	2.3 × 10 ⁷ (2.4 × 10 ⁷)	0.177	7.7 × 10 ⁷ (4.4 × 10 ⁷)	5.7 × 10 ⁶ (5.1 × 10 ⁶)	0.007	
	Stomach	1.8 × 10 ⁶ (1.6 × 10 ⁶)	1.0 × 10 ⁶ (1.2 × 10 ⁶)	0.429	6.3 × 10 ⁵ (9.5 × 10 ⁵)	4.6 × 10 ⁵ (4.8 × 10 ⁵)	0.729	5.6 × 10 ⁵ (4.9 × 10 ⁵)	1.8 × 10 ⁵ (2.7 × 10 ⁵)	0.167	
	Jejunum	3.3 × 10 ⁶ (2.8 × 10 ⁶)	1.4 × 10 ⁶ (1.8 × 10 ⁶)	0.240	7.0 × 10 ⁵ (3.8 × 10 ⁵)	5.4 × 10 ⁵ (6.0 × 10 ⁵)	0.633	1.7 × 10 ⁶ (1.9 × 10 ⁶)	2.6 × 10 ⁵ (2.6 × 10 ⁵)	0.127	
56	Colon	1.6 × 10 ⁷ (4.3 × 10 ⁶)	7.2 × 10 ⁷ (1.4 × 10 ⁸)	0.389	3.3 × 10 ⁶ (1.5 × 10 ⁶)	2.4 × 10 ⁷ (4.1 × 10 ⁷)	0.289	5.5 × 10 ⁶ (2.8 × 10 ⁶)	3.4 × 10 ⁶ (5.4 × 10 ⁶)	0.474	

Piglets in the probiotic group showed significantly ($p \leq 0.05$) lower total *Enterococcus* spp. cell numbers on day 14 in colon contents and on day 35 in jejunum and colon contents. Total *Enterococcus* spp. concentrations in the stomach on day 28 tended to be lower in the probiotic group ($p \leq 0.1$; Table 1). Significant differences in *E. faecalis* cell counts were closely related to total enterococci cell counts, namely on day 14 (colon), day 28 (stomach) and day 35 (jejunum, colon) (Table 1). Thus, it is likely that the reduction of total enterococci is a result of reduced *E. faecalis* cell numbers, i. e. at least the magnitude of the reduction correlates well with the decrease in cell numbers of total enterococci. Additionally, *E. faecalis* cell counts for stomach and jejunum were slightly lower in the probiotic group on day 14 ($p \leq 0.1$). Since *E. faecalis* cell numbers were not different in mother sow feces 10 days ante partum, it is unlikely that the total amount of fecal *E. faecalis* cells affected the cell counts in suckling piglets. Strain composition may be of more importance, i.e. *E. faecalis* strains with less colonization potential for young piglets were present in feces of the probiotic treated sows. Total *E. faecium* cell numbers were not significantly different between treatment groups (Table 1).

As for the detection of the probiotic *E. faecium* strain in young piglets, the observed differences in cell numbers of *E. faecalis* on day 14 in suckling piglets can not be related to ingestion of probiotic supplemented prestarter feed, which was offered only from day 15 on. In addition, studying identical piglets our research group on probiotics in pigs has shown in other reports on physiology (Lodemann *et al.*, 2006), microbiology (Pollmann *et al.*, 2005; Scharek *et al.*, 2005; Taras *et al.*, 2006) and immunology (Scharek *et al.*, 2005) that the influence of the probiotic *E. faecium* on the determined parameters was also clearly visible already on day 14. Therefore, as mother sows received a diet supplemented with the probiotic strain and the probiotic is present in feces of mother sows (average of 1.8×10^5 colony forming units per gram feces) it is conceivable that suckling piglets were inoculated by contact with sow feces. According to Sansom and Gleed, 1981, suckling piglets consume approximately 20g feces per day, with intake ranging from about 6 to 86 g/d. These interindividual variations of feces intake among piglets may offer part of an explanation for the high differences of all recorded *Enterococcus* cell counts between animals throughout the feeding trial. The preweaning intake of sow feces occurs both, directly by voluntary uptake from the pen floor as well as indirectly during suckling on a sow udder coated with feces. Concerning the variation within a litter, it is reasonable to assume that the hierarchy between suckling piglets will determine the major route and total amount of intake of sow feces. Satiety (i.e. the ability to reach the mother teat) will result in less activity and thus less contact with sow feces. However, the temperament and individual predisposition to "dirtiness" of the sow should be taken into account, which is responsible for the majority of the interlitter variation of feces intake by piglets (Sansom and Gleed, 1981). In contrast, within a litter lightweight piglets with less milk intake or shorter accession times to the udder (Pajor *et al.*, 1999; Broom, 1983; Kuller *et al.*, 2004) may be more active and consume higher amounts of creep feed as well as more sow feces directly from the

pen floor. The influence of the microbial feces composition of sows is not limited on the intestinal *Enterococcus* spp. of the piglets as recent works have shown. In all young mammals, e. g. human infants, nursing mice and piglets alike, the development of the intestinal microbiota depends on their environmental conditions, especially the composition of the maternal microbiota (Tannock *et al.*, 1990a; Mackie *et al.*, 1999; Akkermans *et al.*, 2000; Ley *et al.*, 2005; Tannock *et al.*, 1990b; Bateup *et al.*, 1998; Dritz, 2002). Therefore, the priming effect during early life may well be related to the total amount of maternal and piglet feces consumed. Intestinal bacteria from the mother sow may thus have a larger influence on the bacterial development of active piglets, while the initial microbiota of less active piglets could be determined by sow milk.

Conclusions

The results of this study confirm that the probiotic *E. faecium* NCIMB10415 is transferred to the suckling piglet by the maternal fecal microbiota. However, in view of unmodified enterococcal populations in mother sow feces ten days ante partum, reductions of total *Enterococcus* spp. and *E. faecalis* cell numbers in 14 day old piglets must have been generated in the intestine of the piglet. However *E. faecium* NCIMB10415 was not able to maintain its high proportion within the *Enterococcus* spp. population in post weaning piglets.

Experimental procedure

Animals, housing and diets

Lactating sows (Landrace \times Duroc) were housed individually on straw bedding together with their litters. Piglets were weaned at 28 days of age and reared as pairs or triplets in flat-deck batteries. Basal diets of sows comprised mainly barley and wheat in pelleted form, while the basal diets for piglets were based on wheat and soybean meal in mesh form. Feed was offered twice daily to sows, nursed piglets had *ad libitum* access to prestarter feed from 15 to 28 days of age, weaned piglets were fed *ad libitum*. Water was available *ad libitum* for all animals.

Supplementation of basal diets with the probiotic started 24 days after mating for gestating sows of the probiotic group. All other probiotic feed stuffs for the different feeding phases were supplemented from the start of the respective phase. The mean concentration (\pm SD) of the supplemented *E. faecium* strain NCIMB10415 in feed of gestating sows, lactating sows, nursed piglets and weaned piglets was $1.6 (\pm 1.2) \times 10^6$, $1.2 (\pm 0.7) \times 10^6$, $1.7 (\pm 1.9) \times 10^5$ and $2.0 (\pm 1.3) \times 10^5$ viable cells/g feed, respectively.

Sampling

Fecal samples from lactating sows ($n=5$ per trial group) were taken directly from the rectum 10 days ante partum. After euthanasia, samples of suckling and weaned piglets were taken on day 14 (before access to starter diet), on day 28 (before weaning), on day 35 (one week after weaning) and on day 56 (fattening period). One piglet per litter ($n=5$ per trial group) was chosen for euthanasia.

Total nucleic acids in digesta (1g) of stomach, distal jejunum and colon ascendens were extracted by use of guanidinisoithiosulfate, bead beating with glass

beads (\varnothing 0.3–0.5 mm), subsequent phenol/chloroform extraction and isopropanol precipitation. Crude extracts were purified to PCR grade DNA with commercial silica gel spin columns (NucleoSpinKit Tissue, Machery-Nagel, Dueren, Germany).

Bacterial strains used and generation of calibration extracts

DNA extracts of the following strains were used to evaluate the specificity of the realtime PCR assay: *Bifidobacterium adolescentis* DSM20083, *B. thermophilum* DSM20210, *B. suis* DSM20211, *B. longum* DSM20219, *B. bifidum* DSM20456, *Enterococcus cecorum* DSM20682, *E. faecalis* DSM6134, *E. faecalis* DSM8629, *E. faecalis* DSM13591, *E. faecalis* DSM20376, *E. faecalis* DSM20380, *E. faecalis* DSM20478, *E. faecium* DSM2146, *E. faecium* DSM2918, *E. faecium* DSM6177, *E. faecium* DSM20477, *E. faecium* isolate 333, *E. faecium* isolate 378, *E. faecium* isolate 380, (Institute for Medical Microbiology, Benjamin Franklin hospital, Berlin), *E. faecium* isolate 32 – 39 (Institute for Medical Microbiology, Benjamin Franklin hospital, Berlin), *E. faecium* NCIMB2702, *E. faecium* NCIMB10415, *E. faecium* NCIMB12412, *E. faecium* NCIMB12494, *E. faecium* NCIMB700583, *E. faecium* NCIMB702711, *E. faecium* NCIMB702822, *E. villorum* DSM15688, *Escherichia coli* DSM2840, *Lactobacillus acidophilus* DSM20079, *L. alimentarius* DSM20249, *L. animalis* DSM20602, *L. bifementans* DSM20003, *L. bifidus* DSM20100, *L. brevis* DSM20054, *L. bulgaricus* DSM20081, *L. casei* DSM20011, *L. crispatus* DSM20584, *L. delbrueckii* DSM20711, *L. farciminis* DSM20184, *L. fermentum* DSM20052, *L. gallinarum* DSM10532, *L. jensenii* DSM20557, *L. johnsonii* DSM10533, *L. murinus* DSM20452, *L. panis* DSM6035, *L. paracasei* DSM20020, *L. plantarum* DSM 20174, *L. pontis* DSM8475, *L. reuteri* DSM20016, *L. rhamnosus*, DSM20021, *L. salivarius* subsp. *salivarius* DSM20555, *L. sharpeae* DSM20505, *L. vaginalis* DSM5837, *L. zeae* DSM20178, *Lactococcus lactis* subsp. *lactis* DSM20481, *S. alactolyticus* DSM20728, *S. bovis* DSM20480, *S. dysgalactiae* subsp. *dysgalactiae* DSM20662, *S. gallolyticus* DSM13808, *S. hyointestinalis* DSM20770, *S. infantis* DSM 12492, *S. mutans* DSM20523, *S. porcinus* DSM20725, *S. salivarius* DSM 20560, *S. suis* DSM9682, *Weissella confusa* DSM20196 and *W. hellenica* DSM7378.

The following strains were used to create calibration extracts in autoclaved sow feces: *E. faecium* DSM2146,

E. faecium DSM20380, *E. faecalis* DSM6134 and *E. faecalis* DSM20478. Calibration of the probiotic strain *E. faecium* NCIMB10415 was carried out with similar calibration extracts which included 7 other bacterial strains not relevant to this study.

All strains used for calibration extracts were grown 18 hours anaerobically in brain–heart infusion broth at 37°C with shaking. After centrifugation, a wash in 0.85% NaCl and microscopic determination of cell number via Neubauer counting chamber, respective cell numbers were inoculated into 5g autoclaved sow feces (5×10^8 to 5×10^2 cells per sample). Spiked samples were kneaded in plastic bags and triplicate 1g aliquots were taken for DNA extraction. After extraction, respective purified DNA solutions were combined.

Real time PCR assays

Primer sequences are given in Table 2. All primers were purchased from MWG Biotech (Straubing, Germany). A stratagene MX3000p (Stratagene, Amsterdam, The Netherlands) was used for PCR amplification and fluorescent data collection. The supplied software (MxPro V 3.0) was used to calculate PCR amplification efficiency as well as cell numbers according to the calibration curves. The mastermix consisted of 12.5 μ l Brilliant SYBR Green QPCR Mastermix (Stratagene, Amsterdam, The Netherlands), 0.5 μ l of each primer (10 μ M), 0.75 μ l ROX reference dye (1:500 diluted) and 10.75 μ l water. A 1 μ l sample was added before PCR amplification. PCR products with correct melting temperature profiles were randomly (n=10 per primer pair) checked by agarose gel electrophoresis (2%).

Statistical analysis

Differences between means were detected by the ttest. One factorial ANOVA procedures and test for linearity were carried out after elimination of outliers to compare the effect of the probiotic *E. faecium* strain on total *Enterococcus* spp., *E. faecium* and *E. faecalis* cell counts on each sampling day and intestinal segment. Level of significance for all statistical procedures was set to $p \leq 0.05$.

Acknowledgement

This study was part of the program of the German Research Foundation research group FOR438.

Name	Specificity	Sequence 5`-3`	Reference
Ent-F	<i>Enterococcus</i> spp.	CCCTTATTGTTAGTTGCCATCATT	Rinttilä <i>et al</i> , 2004
Ent-R		ACTCGTTGTACTTCCCATTGT	
efaAfm-1	<i>E. faecium</i>	CTTATGATTTGCCAGCAGCA	Eaton and Gasson, 2001
efaAfm-2		TGGATTGTTTCGATGTTCCA	
efaAfs-1	<i>E. faecalis</i>	TTCACTGGCTACCTGCTGTG	Eaton and Gasson, 2001
efaAfs-2		AACGCGCCAATTTGTTTTAC	
Cyl-1	<i>E. faecium</i> NCIMB10415	TCG GAA TTT GCC AGA AGA AC	This study
Cyl-2		CTG GTG AAG CAG GGT TTC AT	This study

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