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EFFECT OF PROBIOTIC OR ANTIMICROBIAL TREATMENTS ON THE INTESTINAL BACTERIAL COMMUNITY IN PIGS

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SUMMARY

The aim of this study was to observe the effect of a probiotic, *Pediococcus acidilactici*, or an antibiotic, amoxicillin, on the diversity and structure of the commensal dominant bacteria and Archaea at various levels of the pig intestinal tract. Forty-eight Specific Pathogen Free pigs (SPF), 70 days old, were separated into 3 experimental groups at day 0 (D0) i) the negative controls i.e. pigs receiving no product, ii) pigs fed with a diet containing the probiotic tested, and iii) the positive controls including pigs treated with amoxicillin. The antibiotic was used at 0.1g/kg of pig weight, once a day during 3 days (from D42 to D44). The probiotic, *Pediococcus acidilactici*, at 1g (10^{10} CFU)/kg of feed, was given from D14 to D44. The digestive microbiota was studied by a molecular method, Capillary Electrophoresis-Single Strand Conformation Polymorphism (CE-SSCP). Using this fingerprint technique, we investigated the total bacterial population in the ileum, caecum, colon, as well as the *Bacillus-Streptococcus-Lactobacillus* (BSL) group and Archaea in caecum and colon. Probiotic decreased bacterial diversity in ileum whereas antibiotic decreased bacterial diversity in caecum and colon. Antibiotic had a significant effect on the total bacterial structure of caecum, colon and ileum. The effects of both treatments on total bacterial structure were highest in the caecum. The effect of both treatments was higher on BSL structure in colon than in caecum. The archeal structure in caecum and colon remained very stable whatever the treatment.

INTRODUCTION

Antibiotic growth promoters (AGP) have been used the past decades in animal production, introducing the potential risk for a reservoir in food animals of antibiotic resistant bacterial populations transmittable to humans [1, 2]. Therefore the European Union moved towards a ban of AGP. As a consequence, alternative feed additives were used to improve animal health and performance, such as probiotics, thought to establish a balanced gut microflora [3, 4]. This study aimed to look at the effect of one probiotic, *Pediococcus acidilactici* on the diversity and structure of commensal microorganisms at different levels of the pig intestinal tract (ileum, caecum, colon) using Capillary Electrophoresis-Single Strand Conformation Polymorphism (CE-SSCP) targeting all bacteria, the *Bacillus-Streptococcus-Lactobacillus* (BSL) group and Archaea. In this study, the antibiotic treatment, administered at a therapeutic dose, was used as a positive control since such a treatment is known to induce changes in the intestinal microbiota.

MATERIAL AND METHODS

Forty-eight SPF pigs of our local flock were separated randomly into 3 experimental groups at D0, i) the negative controls i.e. pigs receiving no product, ii) pigs fed with a diet containing the probiotic tested, from D14 up to D44 (*Pediococcus acidilactici* CNCM MAI 18/5M, at 1g/kg of feed, i.e. 10^{10} CFU/kg of feed), and iii) the positive control being pigs treated with an antibiotic, (amoxicillin at 0,1g/kg of animal weight, orally administered once a day, 3 consecutive days, from D42 to D44), in order to compare changes due to probiotic *versus* antibiotic.

The pigs, 70 days old at the beginning of the study (D0), were fed *ad libitum* a diet for growing pigs, examined daily for rectal temperature, feces aspect, feed refusal and weighed weekly. Samples of contents of colon, caecum and ileum were collected during autopsies and stored at -20°C until DNA extraction. DNA was extracted from 200 mg of each sample using a QIAamp®DNA stool mini-kit according to the manufacturer's instructions. Amplicons of the 16SrDNA (V3 region) were obtained using fluorescent primers w49 and w104, specific of the Eubacteria phylogenetic domain [5]. An additional nested PCR was performed for the analysis of the *Bacillus-Streptococcus-Lactobacillus* (BSL) and archeal communities, using universal primers w108 and w96 respectively [5, 6]. The CE-SSCP was then performed on an ABI Prism 3130 analyzer with the PCR products obtained previously. Statistical analyses were carried out using a statfingerprint / R software. The Simpson diversity index of CE-SSCP profiles was estimated ($S = 1 - \sum P_i^2$, where P_i is the proportion of the peak area). The community structure of the ecosystem was defined as the number of strains (number of peaks) and their relative abundance (comparison of the peak size for each scan of the profile). The ANOSIM-R value indicated the extent to which the groups differed. R close to 1 indicates separated groups. The closer R gets to 1, the more groups are separated, whereas R values close to 0 indicate overlapping groups [7].

RESULTS

No morbidity / mortality was recorded during the survey and no significant differences were observed on the weekly relative weight gain and feed intake of the pigs whatever the treatment.

The dynamics of the bacterial communities in the pig intestinal tract was evaluated by the diversity index and the degree of similarity.

1) Comparison between intestinal contents.

In control pigs, the ileum bacterial diversity was significantly lower than that of caecum and colon (table 1; all p values < 0.005). The CE-SSCP profiles showed a change in bacterial community structures within the 3 intestinal segments.

2) Effect of treatments on the total bacterial structure.

The probiotic had a significant negative effect on the bacterial diversity of the ileum and not on the other compartments (table 1). On the opposite, the antibiotic had a significant negative effect on the bacterial diversity of caecum and colon but not in the ileum (table 1).

Considering the degree of similarity R between CE-SSCP profiles, the antibiotic had a significant effect on the total bacterial structure of caecum, colon and ileum, whereas the probiotic had a significant effect only in the caecum and colon (table 1). The effects of both treatments on total bacterial structure were highest in the caecum.

3) Effects of treatments on *Bacillus-Streptococcus-Lactobacillus* (BSL) and archeal communities

The dominant BSL group, mainly found in the colon, had its structure significantly affected by the antibiotic in both caecum and colon, whereas an effect of probiotic was only observed in the colon (table 2).

No significant effect was observed on the archeal community whatever the treatment.

DISCUSSION

The CE-SSCP method was successful in assessing changes in the microbial diversity of the intestinal microbiota but this method did not allow the identification of specific strains. Our results showed that the bacterial diversity indexes were very similar between colon and caecum whereas the bacterial community structures varied within the 3 intestinal contents. Considering more specific bacterial populations, no significant difference of the BSL community was observed between caecum and colon, which is in agreement with a previous study [8]. The effect of both treatments on the dominant bacterial structure was mainly observed in caecum, whereas it was higher in colon for the changes observed for the BSL structure. This could be explained by more sensitive BSL strains in the colon where the antibiotic effect was about 2.6 higher than that of the prebiotic.

CONCLUSIONS

Although the CE-SSCP method proved to be effective in assessing microflora dynamics in the intestinal contents of growing pigs, further investigations will be needed to identify the bacteria affected (positively or negatively) by the treatments. It would be also interesting to observe the effect of *Pediococcus acidilactici* on piglets with bacterial infections, since probiotic therapy has already made its way in the treatment of various infections.

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TABLE 1: Effects of antibiotic and probiotic treatments on the bacterial diversity of the intestines

Intestinal content	Treatment	Diversity Index	SD	Significance (p) (T vs control)	Degree of similarity R (T vs control)	Significance of R (p)
Ileum	Control	5.3	0.4	/	/	/
	Antibiotic	5.3	0.4	> 0.05	0.122	0.04
	Probiotic	4.8	0.3	0.014	0.075	> 0.05
Caecum	Control	6.2	0.5	/	/	/
	Antibiotic	5.7	0.3	0.016	0.463	0.01
	Probiotic	6.5	0.2	> 0.05	0.205	0.01
Colon	Control	6.6	0.3	/	/	/
	Antibiotic	5.9	0.3	0.0002	0.397	0.01
	Probiotic	6.5	0.4	> 0.05	0.172	0.01

T, treatment; Diversity index was expressed as the mean of 8 individual indexes \pm SD (Standard Deviation).

TABLE 2: Effects of antibiotic and probiotic treatments on the BSL structure

Intestinal content	Treatment	Degree of similarity R (T vs control)	Significance of R (p)
Caecum	Antibiotic	0.378	0.002
	Probiotic	0.069	> 0.05
Colon	Antibiotic	0.467	0.001
	Probiotic	0.179	0.03