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Short communication

A comparison of microbial profiles of different regions of the equine hindgut

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ABSTRACT

The microbial profiles of the luminal content of five hindgut segments of one healthy horse were compared with rectal sample to elucidate the effect of anatomical region on bacterial and archaeal community structure and to evaluate the use of faeces as a representative model of large intestine. The qualitative and quantitative changes of the microbial community composition of caecum, right ventral colon, left ventral colon, left dorsal colon, right dorsal colon and faeces were monitored by denaturing gradient gel electrophoresis (DGGE) and real-time PCR using universal primers amplifying the V3 region of 16 S rDNA. DGGE fingerprints revealed extensive bacterial as well as archaeal diversity in all studied samples and reflected shifts in the community structure among the caecum, the different segments of the colon and the faeces. Archaeal DGGE pattern of the caecum differed from all the other parts of the hindgut. Microbial profile similarities were found between the left and the right dorsal colon and between the left ventral colon and the faeces. The excised DGGE bands were related to uncultured bacteria and methanogens, the dominant archaeal bands of caecum and faeces were related to *Methanocorpusculum* sp. Diversity indices indicated the higher diversity for bacteria than for archaea and the dominance of some methanogenic species. The real-time PCR revealed the differences in the microbial quantitative composition of each segment, showing the highest number of total bacteria and archaea in the right ventral colon. The analyses of bacterial and archaeal composition along the one equine hindgut indicate that the faecal sample is similar to that of the left ventral colon, but does not represent the microbial community of the caecum and other parts of the colon.

1. Introduction

Horses are non-ruminant, hindgut fermenting herbivores adapted to eat large quantities of high fiber diet to obtain energy and nutrients, which are necessary for their metabolism. The equine large intestine is immensely enlarged fermentative chamber, which contains an extremely abundant and diverse community of anaerobic microorganisms. The microbial end-products, especially acetate, propionate and butyrate, absorbed through the intestinal wall and transported in the blood to the liver and tissues, supply the horse body by energy. The intestinal microbial community of similar complexity as ruminants consisting of eubacteria, archaea, anaerobic fungi ciliates and bacteriophages (Sadet-Bourgeteau and Julliand, 2010) resides in big caecum and voluminous colon and is assumed to contribute from 50% (Argenzio, 1975) up to 68% (Vermorel and Martin-Rosset, 1997) to total energy by producing SCFA. Caecum alone can provide even 30% of energy (Glinsky et al., 1976). This high amount of energy retreaved by the horse from the end microbial fermentation products points out the significance of equine caeco-colic microbiota, which however, compared to ruminants, has received much less attention.

The equine bacterial population shown dominated by Firmicutes and Bacteroidetes has been studied mostly in feaces (Yamano et al., 2008; Shepherd et al., 2012; Costa et al., 2012; Steelman et al., 2012; Fernandes et al., 2014; Dougal et al., 2014). Equine archaeal population has been analysed only in faeces and *Methanocorpusculum labreanum* and *Methanobrevibacter ruminantium* have been detected as the predominant species (Lwin and Matsui, 2014; Fernandes et al., 2014). Faeces are commonly used for microbial investigation of horse large intestine, because they provide the ethical noninvasive sampling and are generally accepted as representatives of equine gut microbiome. However, increasing numbers of results indicate that gut region appears to influence the abundance and composition of equine intestinal bacteria (Daly et al., 2001, 2012, de Fombelle et al., 2003; Hastie et al., 2008;

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Dougal et al., 2011, 2013; Schoster et al., 2013; Sadet-Bourgeteau et al., 2014; Costa et al., 2015). Significant differences have been observed especially between colon and feaces, but the microbial comparison of samples from caecum, ventral colon, dorsal colon and rectum has also indicated the population variance along the lower hindgut. The role of pelvic flexure in the bacterial community shift seems to be more and more consensual. The aim of the present study was to analyse the microbial community profile in different parts of one healthy horse digestive tract to elucidate the effect of anatomical region on bacterial and archaeal intestinal diversity and evaluate whether faeces can serve as a suitable model of large intestine. The microbial composition of luminal content of five segments of equine gastrointestinal tract (caecum, right ventral colon, left ventral colon, left dorsal colon and right dorsal colon) was compared with rectal sample by population fingerprinting using PCR-DGGE analysis of 16 S rRNA gene amplicons and the relative microbial quantification using the real-time PCR method.

2. Material and methods

2.1. Animal and diet

The samples for this study were taken from one adult (24 years old) Anglo-Arabian castrated male horse reared in Sardinia (Italy). The horse (410 kg live-weight) housed in stall bedded with wood shavings, regularly vaccinated and dewarmed, was offered by meadow hay (5.4 kg of DM/day) and concentrate (1.6 kg of DM/day) devided into 2 meals. The composition of the concentrate is reported in the Table S1. The horse euthanized for nonresearch reasons was clinically healthy and did not show any clinical sign of disease or intestinal disturbances within the previous 6 months.

2.2. Sample collection and DNA extraction

The samples of the luminal gut contents were taken from three different regions of the large intestine: caecum (C), colon (I – IV) and rectum (F). In particular, from the colon the samples of right ventral colon (RVC, I), left ventral colon (LVC, II), left dorsal colon (LDC, III) and right dorsal colon (RDC, IV) have been taken. The samples collected immediately after the euthanasia were placed into a clean bag under vacuum seal, transported on ice in the laboratory, and freeze-dried. Nucleic acids were extracted from 400 mg of intestinal sample disintegrated into powder using the modified method of Gardes and Bruns (1993).

2.3. DGGE analysis of bacteria and archaea

Amplicons for the study of bacterial and archaeal profiles in different parts of one horse digestive tract were prepared by nested PCR approach according to Muyzer et al. (1993) using the primer sets listed in the Table S2. The DGGE electrophoresis, gel analysis and identification of bands of interest were performed as described previously by Mrázek et al. (2008). BioNumerics program (Applied Maths, U.S.A) was used for the calculation of the species evenness and Shannon index.

2.4. Real-time PCR analysis of bacteria and archaea

The relative quantification of the total bacteria and archaea was performed according to Bartosch et al. (2004) and Ovreas et al. (1997), respectively, with MX 3005P QPCR System (Stratagene, U.S.A), using the Kapa SYBR Fast qPCR Master mix (Kapa Biosystems, U.S.A). The caecum sample (C) was chosen as a calibrator and the quantification in the other samples was performed as relative ratio of the detected cycle threshold (C_t). The unpaired *t*-test (Microsoft Excel 2010) was utilised to identify significant differences in the bacterial and archaeal counts among all the samples. Significant differences were declared when $P < 0.01$.

3. Results

3.1. DGGE analysis of bacteria and archaea

PCR-DGGE study was performed to obtain a preliminary insight and comparison of the diversity of bacteria and archaea in samples from six different parts of one horse digestive tract. The population fingerprinting displayed rich band profiles in all the studied samples revealing differences in the community patterns among caecum, colon, and faeces. Both DGGE profiles (Fig. 1) show pattern subclustering of left and right dorsal colon (III and IV) and left ventral colon with faeces (II and F). The archaeal profile of caecum (C) clustered separately, however it was more closely related to LVC and faeces (II and F) then to the other three parts of colon (I, III, IV), which clustered together. The bacterial profile of the caecum was similar to the first part of the colon (I), but it differed clearly from all the other parts of the lower digestive tract. Shannon index, describing the microbial community diversity, was higher for bacteria than for archaea in all parts of hindgut. The equitability index for archaea indicated the dominance of some methanogenic species (Table S3).

Sequence analysis of the reamplified excised bacterial bands revealed low percentage similarity with *F. succinogenes* (2B, 7B), but the most fragments were related to uncultured bacteria. The dominant archaeal fragments from caecum and faeces were identified as *Methanocorpusculum labreanum* (1A, 3A) and *Methanocorpusculum aggregans* (4A) with a sequence similarity percentage 97, 96, and 94%, respectively. The intensive bands from left dorsal colon (2A) and faeces (5A) represented uncultured methanogenic clones (Table S4).

3.2. PCR quantification

The results of the relative PCR assessment of Eubacteria and Archaea depicted in the Fig. 2 illustrate the considerable differences and fluctuation of the microbial amount along the one horse caeco-colic tract. The quantitative PCR determined the highest archaeal count in the right ventral colon (I) and the significant decrease ($P < 0.01$) in the sequent left ventral colon (II). The lowest amount of methanogens has been detected in the right dorsal colon (IV), but the number of archaea significantly increased ($P < 0.01$) again in the faeces (F). The highest bacterial count determined in the right ventral colon (I) was significantly higher than that in the caecum ($P < 0.01$). The amount of bacteria significantly decreased ($P < 0.01$) in the sequent left ventral colon (II), then their number significantly increased ($P < 0.01$) again in the left dorsal colon (III) and maintained on comparable levels in the right dorsal colon (IV) and faeces (F).

4. Discussion

The caeco-colonic microbial fermentation of digesta in horses is crucial for nutrition and physiology of the host as well as its welfare and behavior. Despite this fact, horse large intestine microbiome has been relatively little studied. Moreover, faeces samples were often utilised and little attention has been given to how closely the microbial population of faeces actually represents the equine large intestine microbiome. This lack of knowledge has been gradually updated by comparative studies showing differences mainly in the bacterial population of horse caecum, colon and faeces (Hastie et al., 2008; Dougal et al., 2012; Schoster et al.,