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Comparative assessment of human and farm animal faecal microbiota using real-time quantitative PCR

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Abstract:

Pollution of the environment by human and animal faecal pollution affects the safety of shellfish, drinking water and recreational beaches. To pinpoint the origin of contaminations, it is essential to define the differences between human microbiota and that of farm animals. A strategy based on real-time quantitative PCR (qPCR) assays was therefore developed and applied to compare the composition of intestinal microbiota of these two groups. Primers were designed to quantify the 16S rRNA gene from dominant and subdominant bacterial groups. TaqMan® probes were defined for the qPCR technique used for dominant microbiota. Human faecal microbiota was compared with that of farm animals using faecal samples collected from rabbits, goats, horses, pigs, sheep and cows. Three dominant bacterial groups (*Bacteroides/Prevotella, Clostridium coccoides* and *Bifidobacterium*) of the human microbiota showed differential population levels in animal species. The *Clostridium leptum* group showed the lowest differences among human and farm animal species. Human subdominant bacterial groups were highly variable in animal species. Partial least squares regression indicated that the human microbiota could be distinguished from all farm animals studied. This culture-independent comparative assessment of the faecal microbiota between humans and farm animals will prove useful in identifying biomarkers of human and animal faecal contaminations that can be applied to microbial source tracking methods.

Keywords: quantitative PCR; faecal microbiota; human; farm animals

Introduction

Faecal pollution in coastal or fresh waters leads to human disease and economic losses such as closure of commercial shellfish-harvesting and recreational and bathing areas. Recent incidents include the isolation of human enteric viruses and bacteria such as norovirus, hepatitis A virus, and *Salmonella* from coastal waters and shellfish which were implicated in shellfish-borne outbreaks after oyster consumption (Potasman *et al.*, 2002, Martinez-Urtaza *et al.*, 2004). In light of this risk to health and safety, it is important to identify the source of faecal contamination to better facilitate resource management and remediation.

Faecal contamination of water resources is currently evaluated by employing culturing methods to detect and enumerate living facultative-anaerobic bacteria, such as *Escherichia coli*, enterococci, or faecal coliforms. Samples are normally obtained from shellfish or directly from bathing waters (Directives 2006/113/CE; 2006/7/CE). The species traditionally used as faecal indicators, however, have limitations owing to several factors including **1**) their short survival time in an open-water environment, **2**) their ability to proliferate in soil, sand or sediments absent any point-source faecal contamination, **3**) the low levels of correlation with the actual presence of pathogens, **4**) the underestimation of true bacterial presence through omission of non-cultivable bacteria, **5**) their inability to track the source of faecal contamination because coliforms and enterococci are common to all mammalian hosts (Roszak & Colwell, 1987; Pommepuy *et al.*, 1996; Gordon & Cowling, 2003; Wheeler *et al.*, 2003; Hörman *et al.*, 2004; Savichtcheva & Okabe, 2006). In order to overcome these shortcomings, alternative methods and indicators must be developed. Potential alternative indicators of faecal contamination could be anaerobic bacteria such as *Bacteroides* and *Bifidobacterium* which are more abundant in the faeces of warm-blooded animals than *E. coli* (Fiksdal *et al.*, 1985; Suau *et al.*, 1999). Importantly, these species have been shown to exhibit host-specific adaptation on the genetic level (Dick *et al.*, 2005). While these bacteria are

fastidious to enumerate with conventional culture techniques, they can nonetheless be easily detected using current molecular methods. Since uncultivated bacteria represent 70 to 80% of the total human microbiota, culture-independent methods of analysis based on 16S ribosomal RNA have been developed (Suau *et al.*, 1999; Eckburg *et al.*, 2005). These studies showed that the most highly represented bacterial groups in human stools were the *Clostridium leptum* and the *Clostridium coccoides* groups of the Firmicutes followed by the *Bacteroides/Prevotella* group and the *Bifidobacterium* genus (Harmsen *et al.*, 2002; Lay *et al.*, 2005a). Studies involving domestic animal microbiota are less numerous and are mainly focused on the phylogenetic diversity of the intestinal bacterial community in pigs, cattle and chicken (Lan *et al.*, 2002; Leser *et al.*, 2002; Ozutsumi *et al.*, 2005). Recently, specific qPCR approaches were used to estimate a limited number of bacterial species or groups of faecal microbiota (Matsuki *et al.*, 2004, Seurinck *et al.*, 2005, Reischer *et al.*, 2006).

The work presented here seeks to establish a more comprehensive dataset in comparing human and farm animal microbiota. To this end, we developed and optimized a qPCR-based approach which was subsequently applied to analyze faecal samples collected from humans and farm animals. Using such molecular techniques we overcome the limits of traditional faecal indicators, including culturing methods, which consistently underestimate faecal population. The development and application of our qPCR systems quantifies faecal bacteria groups in human and animal faecal samples and provides essential information concerning potential alternative faecal indicators and host-specific bacterial groups.

Materials and Methods

DNA extraction from faecal samples

The DNA extracts from faecal samples of 21 human stools were prepared as previously described (Godon *et al.*, 1997, Lay *et al.*, 2005b)**.** Faecal samples from five individual animals

were collected for each of six farm species (rabbit, goat, horse, pig, sheep and cow) and stored at -80°C immediately after sampling. Total cellular DNA was extracted from 0.2 g of animal faecal material using the G'NOME® kit (BIO 101, La Jolla, CA) with modifications. Faecal samples were homogenized in the supplied Cell Suspension Solution. Cell Lysis/Denaturing Solution was then added and the samples incubated at 55 °C for 2 hours. To improve cellular lysis, 750 µl of 0.1-mm-diameter silica beads were added, and agitation carried out at maximum speed for 10 minutes in a Beadbeater (Biospec, Bartlesville, OK). Polyvinylpolypyrrolidone (15 mg) was added to ensure removal of polyphenol contamination that could inhibit subsequent qPCR reactions. Samples were vortexed and centrifuged at 20,000 × *g* for 3 minutes and the supernatant was recovered. The remaining pellet was washed with 400 µl of TENP (50 mM Tris (pH8), 20 mM EDTA (pH8), 100 mM NaCl, 1 % polyvinylpolypyrrolidone) and centrifuged at 20,000 × g for 3 minutes. The washing step was repeated once more and the resulting supernatants pooled. Nucleic acids were precipitated by addition of 1 volume isopropanol, storage at -20°C for 20 minutes, and centrifugation at 20,000 × g for 10 minutes. The pellet was resuspended in 400 µl of distilled water plus 100 µl of Salt-Out Mixture and incubated at 4°C for 10 minutes. Samples were spun for 10 minutes at maximum speed, and the supernatant containing the DNA was transferred to a clean 1.5ml microcentrifuge tube. DNA was precipitated with 2 volumes of 100% Ethanol at room temperature for 5 minutes followed by centrifugation at 16,000 × g for 5 minutes. DNA was resuspended in 150µl of TE Buffer. DNA solutions were stored at -20°C for later analysis.

Validation of the G'NOME DNA extraction method

We compared our DNA extraction method to our former reference (Godon *et al.*, 1997). Two series of DNA extracts from 12 human faecal samples were prepared by each method. The

All-Bacteria primers (Table 1) were used to perform PCR to compare both DNA extraction protocols and to validate our method.

Performance of the real-time qPCR protocol in artificial mixtures.

To validate the performance of our modified G'NOME DNA extraction protocol and to facilitate real-time qPCR methods, we employed an approach whereby individual samples were spiked with a measured quantity of a known bacterial species. Briefly, several tubes (1 ml) of pure culture *Lactococcus lactis* were centrifuged. Pelleted cells were either stored pure at -80°C or used to spike otherwise *Lactococci*-free faecal samples before storage. Total bacterial DNA from 6 pellets and 12 spiked faecal samples was extracted. The resulting levels of *Lc. lactis* were assessed by real-time qPCR using species-specific 16S rDNA primers (Llac05-F: AGCAGTAGGGAATCTTCGGCA and Llac02-R: GGGTAGTTACCGTCACTTGATGAG). The quantitative results from bacterial pellets and spiked faecal samples were compared to validate the performance of our protocol.

Oligonucleotide primers and probes

TaqMan® qPCR was adapted to quantify total bacteria population in addition to the dominant (>1% of faecal bacteria population) bacterial species *C. coccoides, C. leptum, Bacteroides/Prevotella* and *Bifidobacterium*. Quantitative PCR using SYBR-Green® was performed for the sub-dominant bacterial species *Escherichia coli, Streptococcus salivarius,* for the previously described *Enterococcus* group, and for the *Lactobacillus/Leuconostoc/Pediococcus* group. Primers and probes used in this study (Table 1) were designed based on 16S rRNA sequences (EMBL database) aligned with the program Clustal W (Thompson *et al.*, 1994)**.** Primer design was carried out using Primer-Express version 2.0 (Applied-Biosystems). The specificity of the primers and probes was tested by

submitting the sequences to the Probe Match program (Ribosomal Database Project II; Maidak *et al.*, 2001). Prior to laboratory testing, OligoCheck (http://www.cf.ac.uk/biosi/research/biosoft) was used to examine the *in silico* performance of the PCR systems against 5127 sequences of 16S rDNA from type-strains of intestinal bacteria. The TaqMan® probes were synthesized by Applied-Biosystems Applera-France. Primers were purchased from MWG (MWG-Biotech AG, Ebersberg, Germany). Primer and probe specificities were further assessed using the real-time qPCR protocol against a series of selected cultured strains (Table 3).

Real-time qPCR

Real-time qPCR was performed using an ABI 7000 Sequence Detection System with software version 1.2.3 (Applied-Biosystems). Amplification and detection were carried out in 96-well plates with TaqMan® Universal PCR 2X Master Mix (Applied-Biosystems) or with SYBR-Green® PCR 2X Master Mix (Applied-Biosystems). Each reaction was run in duplicate in a final volume of 25 µl with 0.2 µM final concentration of each primer, 0.25 µM final concentration of each probe and 10µl of appropriate dilutions of DNA samples. Amplifications were carried out using the following ramping profile: 1 cycle at 95°C for 10 min, followed by 40 cycles of 95°C for 30 sec, 60°C for 1 min. For SYBR-Green® amplifications, a melting step was added to improve amplification specificity.

Bacterial strains and growth conditions

The various bacterial strains used to control for the specificity of the primers and probes in this study are shown in Table 3. Bacterial strains were either available in our laboratory collection or were otherwise obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ). Bacteria were cultured aerobically or anaerobically on selective broth

as recommended by DSMZ. For each culture, the total number of bacteria, in terms of colony forming units (CFU), was determined by plating. Aliquots of 1 ml of culture were centrifuged at 12,000 × g for 3 minutes and the bacterial pellets were stored at -80°C prior to use.

Bacterial DNA extraction, standard curves and quantification

Bacterial genomic DNA used to generate standard curves was extracted twice with the Wizard Genomic DNA Purification Kit (Promega) following the manufacturer's instructions. For the quantification of bacterial species and groups, standard curves were generated from serial dilutions of a known concentration of genomic DNA from each species or group. Standard curves were generated by plotting threshold cycles (Ct) versus bacterial quantity (CFU). The total number of bacteria (CFU) was interpolated from the averaged standard curves as previously described (Lyons *et al.*, 2000). When PCR was performed on unknown faecal samples, we used these standard curves to quantify each bacterial population. The lower limit for detection for bacterial enumeration with good precision is 10^6 bacteria per gram of stool.

Normalization of Q-PCR results

In human and animal microbiota, All-bacteria results are presented as the mean of the log10 value ± SEM. To overcome the fact that faecal samples may contain more or less water, we have normalized the data for each faecal sample. The level for each bacterial species or group was subtracted by the level of All-bacteria content. The data are given as the log no. of bacteria per gram of faecal sample.

Statistics

In comparing the human microbiota to those of animals, a one-way ANOVA test was performed using JMP® software (Abacus Concepts, Berkeley, CA). When ANOVA indicated

a significant result, values were subsequently compared using non-parametric tests (Wilcoxon). Statistical significance was accepted at $P<0.05$ (P value adjustment method: Holm). Partial Least Squares (PLS) regression was also used (Moulin-Schouleur *et al.*, 2006) to assess the differences between human and farm animal microbiota (variables *Y*) on the basis of the qPCR results (variables *X*). PLS predictive models using PLS regression were established using the SIMCA software, version 8.1 (UMETRI, Umeå, Sweden). The PLS regression between variables *X* and variables *Y* yielded the PLS components. These components described the variables *X* and explained the variables *Y*. The number of useful PLS components was determined by cross-validation (SIMCA-P 9.0, 2001). The *X*-loadings and the *Y*-loadings were noted *w** and *c*, respectively. Groups of strains were presented as situated on a plane defined by the PLS components. The predictive quality of the model was evaluated using the R^2Y coefficient which corresponded to the proportion of the variance of variables *Y* explained by variables *X*.

Results

Validation and performance of DNA extraction

Total bacteria counts, as measured by qPCR, performed on DNA extractions obtained using the former reference method of Godon (1997) and our modified G'NOME method were highly similar. Total bacteria levels in the two series of DNA preparations were 11.55 ± 0.1 and 11.44 ± 0.1 logs of enumerated bacterial for the Godon and G'NOME methods, respectively, with no statistical difference. This result indicates that the performance of our technique is equivalent to that of Godon (1997).

Performance of the real-time qPCR protocols in artificial mixtures

Population levels of *Lc. lactis* determined using qPCR on *Lc. lactis* bacterial pellets and spiked faecal samples were 9.31 ± 0.35 and 9.05 ± 0.39 logs of bacteria, respectively. No significant difference between the two was observed. This result further confirmed the robust nature of the real-time qPCR assay coupled with our DNA extraction method for quantification of bacterial population levels in faecal samples.

Validation of primers and probes

The specificity of all PCR systems (Table 1) was tested by submitting each oligonucleotide sequence to the Probe Match Program (Ribosomal Database Project II) (Maidak *et al.*, 2001). This program identifies the target species, if any, matching each PCR system (Table 2). The results from a complementary program, OligoCheck details the number and position of any mismatches (Table 2; positions of mismatches are provided in a supplementary Table S1).

We tested the resulting PCR systems specificity against DNA extracted from pure cultures of 48 different strains of bacterial (Table 3). All positive and negative PCR assay results corroborated our *in silico* predictions. For the *Lactobacillus* group, it was not possible to design genus-specific primers since *Leuconostoc* was also detected by the PCR system (Table 3).

Composition of human faecal microbiota assessed by qPCR

For the different targeted bacterial groups, qPCR systems were validated using genomic DNA extracted from the faecal microbiota of healthy human subjects. These results defined a "standard" profile for dominant and sub-dominant groups present in the human intestinal microbiota. Dominant species or groups are defined as those found to represent 1% (- 2.0 log no. of bacteria) or more of the faecal bacteria population. *C. leptum*, *C. coccoides* and *Bacteroides/Prevotella* groups are dominant populations (Table 4). Thus, the *Bifidobacterium*

population, having a value of -2.4, suggests a sub-dominant population of human microbiota. This microbiota profile was subsequently used in comparisons against that of farm animals**.**

Comparison of bacterial populations in stools from human and farm animals

Differences in the bacterial composition of animal stool samples compared to those found in the human faecal microbiota were assessed using qPCR (Table 4). Global One-way ANOVA testing showed significant differences in bacterial compositions between the two groups.

The non-parametric Wilcoxon test was used to reveal whether each Q-PCR system allows for discrimination of the bacterial population of humans and animals. This statistical test can also show how animal microbiota differs from human. The *C. leptum* qPCR system revealed several significant differences between human and horse, cow, goat, and sheep microbiota (Table 4). When comparing results between human and rabbit microbiota for the *C. leptum* group, no significant difference was observed (Table 4).

Although unable to distinguish between the microbiota of human and pig, the *C. coccoides* group qPCR system produced significantly different results for all other animals, with values being higher than that of human (Table 4).

The *Bacteroides*/*Prevotella* group displayed the same type of enrichment as *C. coccoides* for horse, cow, goat and sheep microbiota. Two exceptions were noted however, in rabbit and pig, where no statistically difference with respect to human samples was observed (Table 4).

We also found the *Bifidobacterium* genus to vary significantly in the faeces of horse, cow, sheep and pig compared to human (Table 4). The *Bifidobacterium* population in goat and rabbit faeces were similar in relation to human and showed the lowest normalized data (Table 4).

The *Lactobacillus*/*Leuconostoc/Pediococcus* group failed to discriminate the microbiota of animals and human, with the sole exception being for pig samples. It is important to note that

the targeted lactobacilli population in pig microbiota showed the lowest normalized result (Table 4).

The *E. coli* species qPCR system could distinguish human and animal microbiota except in the cases of goat and sheep. Our study showed that the *E. coli* value in pig microbiota is the lowest (-2.7 log no of bacteria) when compared to those of animals and humans, and was not detected in the faecal samples of rabbit (Table 4). *S. salivarius* species was also not detected in faecal samples of rabbit, in addition to being absent from both sheep and pig. Nevertheless, the results show that *S. salivarius* can be used to distinguish the human microbiota from those of horse, cow and goat (Table 4). *S. salivarius* was more abundant in human faecal samples than in the other faecal samples. The *Enterococcus* species could not be detected in any animal faecal sample in contrast to its presence in human samples (Table 4).

PLS regression analysis based on faecal microbiota composition assessed using real-time qPCR confirmed that the human faecal microbiota could be clearly differentiated from that of farm animals in the 95% probability region (Fig.1A). The first two components of the PLS model explained 85% of the variation of the *Y*-matrix, indicating a good separation of the human group compared to the groups of farm animals. The X-loadings (*w**) corresponding to faecal microbiota quantifications and the Y-loadings (c) corresponding to the human and farm animal groups are presented in Figure 1B. PLS regression analysis demonstrated that the *C. coccoides* group, *Enterococcus* genus and *S. salivarius* species characterize the human faecal microbiota and *Lactobacillus/Leuconostoc/Pediococcus* characterize the pig faecal microbiota.

Discussion

Pollution by human and animal faeces harbouring potential human pathogens represents a serious environmental threat that affects many natural waters. Waters contaminated with

human faeces, in particular, are generally considered to represent a greater risk for human health as they contain human-specific enteric pathogens (Baudart *et al.*, 2000; Koopmans & Duizer, 2004; Godfree & Farrell, 2005). Animals can also serve as reservoirs for numerous enteric pathogens (Hancock *et al.*, 2001; Brown *et al.*, 2004; Cox *et al.*, 2005). Given this complex situation, the ability to accurately track faecal contamination in the environment and identify its origin is of great importance. The key points of such a technique are the choice of reliable and differential faecal indicators and the development of quantitative Microbial Source Tracking methods.

To address these requirements, a robust and reproducible protocol is required to quantify bacterial species and groups in faecal samples originating from different possible contamination sources. Matsuki and colleagues (2004) were the first to apply qPCR, based on 16S rRNA gene quantification, to analyze the diversity of human intestinal *Bifidobacterium*. In our work, employing an optimized protocol, we quantified equivalent numbers of *Bifidobacterium* in human samples, compared with Matsuki *et al*. This corroborative result gave us confidence in expanding the use of the qPCR technique to compare the whole human faecal microbiota to that of animals.

One additional variable that, in some cases, could influence the measurement and comparison of different groups of bacteria is the water content of each faecal sample. Low water content, for example, could contribute to the high bacterial concentration observed in goat and sheep samples. To overcome this potential variable, we normalized our data using All-bacteria populations.

As discussed below, our data are consistent with a number of smaller-scale investigations which focused on individual farm species or targeted groups of bacteria. In our study, we observed that the pig faecal microbiota is characterized by a population of *Lactobacillus/Leuconostoc/Pediococcus* higher than that found in other animals or humans.

Given the value of -1.2 log no. of bacteria, this population could be considered dominate in pig microbiota. These data are in agreement with the observation by Castillo *et al.* (2006) showing a high level of *Lactobacillus* in the upper gastrointestinal tract of pig. These results, combined with those obtained for *E. coli*, suggest that both populations can be considered important in pig microbiota.

Canzi *et al.* (2000) enumerated *Bacteroides* and *Clostridium* in rabbit faeces. We also found the same range of populations for the *Bacteroides*/*Prevotella* group. However, for *Clostridium* populations, our study indicated higher colonization levels (about 6 logs higher) than those observed by these authors. This discrepancy could be due to methodological differences as Canzi *et al.* used spore enumerations for their *Clostridia* estimation. The fact that our technique enumerates vegetative cells as well as non-cultivable bacteria is the most likely explanation for the higher concentration observed. Moreover, our PCR system also detected *Eubacteria* and *Ruminococci* species which are part of the *Clostridium* group.

For equine microbiota, our results are consistent with a previous study (Daly and Shirazi-Beechey, 2003) where the authors used oligonucleotide probes in hybridization assays. Daly and Shirazi-Beechey found no *Bifidobacterium* and observed that the *Eubacterium rectale-Clostridium coccoides* group, combined with *Spirochaetaceae* and the *Cytophaga-Flexibacter-Bacteroides* assemblage, represented the largest colonized populations (10 to 30%). The authors further noted that the *Bacillus-Lactobacillus-Streptococcus* group with *Fibrobacter* constituted 1 to 10% of the total microbiota in horse samples.

It is likely that the bacterial biodiversity of the equine microbiota compared with human contributes to the significant differences in bacterial quantification. Quantitative PCR developed to detect intestinal bacteria in human samples further highlight the species specificity of our protocols and the fact that the bacterial biodiversity of the equine microbiota is notably different from that of human.

Several studies have also reported on the bovine intestinal microbiota. Stahl and co-workers (1988) used species- and group-specific 16S rRNA targeted probes for enumeration of two species (*Fibrobacter succinogenes* and *Lachnospira ruminicola*) in the rumen of animals treated with antibiotics. Tajima (2001) used qPCR to quantify several *Prevotella* and some *Ruminococcus*, *Fibrobacter,* and *Eubacterium* species in the rumen. In 2005, An *et al.* estimated the prokaryote diversity in the rumen of yak (*Bos grunniens*) and Jinnan cattle (*Bos taurus*) by 16S rRNA sequence homology analysis. Their results showed a prevalence of *Bacteroides,* however, no sequence was related to *Ruminococcus albus* (a species of the *C. leptum* group) in the yak and cow rumen. In our study, the level of *Bacteroides/Prevotella* population presents a normalized difference of -2.3 log no. of bacteria and can not be regarded as a dominant population, while *C. leptum* group shows only -1.0 log no. of bacteria and is part of the dominant population. Whitford (1998) and Ozutsumi (2005) presented a phylogenetic analysis of rumen bacteria by comparative sequence analysis of cloned 16S rDNA. Approximately 30% of the sequences were related to bacteria of the *Bacteroides/Prevotella* group, most of which clustered with *Prevotella ruminicola*. The remaining sequences clustered with members of the *Clostridium* genus. The differences observed with our findings are likely due to different technical approaches and/or diversity of microbiota among bovine herds.

To our knowledge, no previous study has used qPCR techniques to describe and compare the intestinal microbiota between animal and human. Our qPCR systems, checked *in silico* by OligoCheck against RDP databases, were successfully able to discriminate different intestinal microbiota.

Our global comparison between human and farm animal microbiota provides data to select host-specific bacterial groups and alternative faecal indicators from all hosts considered.

Our PLS regression analysis showed that *C. coccoides* group, *Enterococcus* genus and *S. salivarius* species could be considered as specific markers for human faecal microbiota and that *Lactobacillus/Leuconostoc/Pediococcus* can be used as a specific marker of pig microbiota.

The *C. leptum* group was found to have the lowest normalized data in humans and animals and thus represents a promising candidate for use as a reliable faecal indicator. It is largely distributed among animal species and in humans and has also been linked with diseases (Manichanh *et al.*, 2006; Sokol *et al.*, 2006). Our study also shows high concentrations of *Bacteroides/Prevotella* and *Bifidobacterium* in all host faecal samples tested. Such anaerobic bacteria do not persist for long periods of time in aerobic waters and are generally unable to multiply in such conditions (Fiksdal *et al.*, 1985; Kreader, 1998). These inherent physiological characteristics make the *Bacteroides* and *Bifidobacterium* excellent candidates for detecting faecal contamination in the environment. Integrated within these two dominant bacterial groups are several species that were found to be host-specific in several studies (Bernhard & Field, 2000a; Bonjoch *et al.*, 2004; Dick *et al.*, 2005). Host-specific *Bacteroides* markers were developed (Bernhard & Field, 2000b; Dick *et al.*, 2005) and applied in a watershed in the USA (Shanks *et al.*, 2006). They were also validated on French faecal and environmental samples (Gourmelon *et al.*, 2007). Quantitative PCR assays are currently in progress and some results have already been published for human and bovine-specific *Bacteroides* (Seurinck *et al.*, 2005; Reischer *et al.*, 2006).

Among the teams who have studied the microbiota of animals over the last decade none, up to now, has presented a global comparison of the faecal microbiota composition of humans and animals. Our results are thus promising in advancing the goal to define a discrete set of host-specific faecal microbiota biomarkers. Additional investigations are continuing to refine a set of comprehensive, reliable, and predictive host-specific markers.

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Table 1: Group and species-specific 16S rRNA-targeted primers and probes used in this study. Probe sequences are in bold.

Target organism	*Primer and probe*	*Sequence 5'- 3'*	*Source of reference*
All-bacteria **(*)**	F_Bact 1369	CGG TGA ATA CGT TCC CGG	(Suzuki *et al.*, 2000)
	R_Prok1492	TAC GG**C** TAC CTT GTT ACG ACT T	
	P_TM1389F	**6FAM-CTT GTA CAC ACC GCC CGT C**	
C. leptum	F_Clept 09	CCT TCC GTG CCG SAG TTA	This study
	R_Clept 08	GAA TTA AAC CAC ATA CTC CAC TGC TT	
	P-Clep 01	**6FAM-CAC AAT AAG TAA TCC ACC**	
Bifidobacterium	F_Bifid 09c	CGG GTG AGT AAT GCG TGA CC	This study
	R_Bifid 06	TGA TAG GAC GCG ACC CCA	
	P_Bifid	**6FAM-CTC CTG GAA ACG GGT G**	
C. coccoides	F_Ccoc 07	GAC GCC GCG TGA AGG A	This study
	R_Ccoc 14	AGC CCC AGC CTT TCA CAT C	
	P_Erec482(*)	**VIC-CGG TAC CTG ACT AAG AAG**	(Franks *et al.*, 1998)
Bacteroides/ Prevotella	F_Bacter 11	CCT WCG ATG GAT AGG GGT T	This study
	R_Bacter 08	CAC GCT ACT TGG CTG GTT CAG	
	P_Bac303(*)	**VIC-AAG GTC CCC CAC ATT G**	(Manz *et al.*, 1996)
E. coli	E.coli F	CAT GCC GCG TGT ATG AAG AA	(Huijsdens *et al.*, 2002)
	E.coli R	CGG GTA ACG TCA ATG AGC AAA	
Lactobacillus/ Leuconostoc/ Pediococcus	F_Lacto 05	AGC AGT AGG GAA TCT TCC A	This study
	R_Lacto 04	CGC CAC TGG TGT TCY TCC ATA TA	
S. salivarius	Stherm 03	TTA TTT GAA AGG GGC AAT TGC T	(Furet *et al.*, 2004)
	Stherm 08	GTG AAC TTT CCA CTC TCA CAC	
Enterococcus	F_Entero	CCC TTA TTG TTA GTT GCC ATC ATT	**(**Rinttilä *et al.*, 2004)
	R_Entero	ACT CGT TGT ACT TCC CAT TGT	

(*) modified from reference

1 Table 2: Bacterial target species for group or species specific primers
2

PCR systems	Target species*
C. leptum group	*Clostridium leptum*$*(1), C. methylpentosum(2), C. sporosphaeroides (2), Faecalibacterium prausnitzii* $*(1).* *Ruminococcus albus*$*(0), R. callidus(0), R. flavefaciens(0), R. bromii(1).* *Others: see TableS1*
C. coccoides group	*Clostridium coccoides*$*(0), C. aerotolerans(3), C. indolis(4), C. algidixylanolyticum(4), C. aminophilum (2), C. aminovalericum(5), C. amygdalium (4), C. bolteae(5), C. celerecrescens(4), C. clostridioforme(2), C. hathewayi(3), C. herbivorans(2), C. hylemonae(2),C. jejuense(2), C. lentocellum(5), C. nexile(2), C. oroticum(7), C. populeti(2), C. proteoclasticum(2), C. scindens(2), C. saccharolyticum(4), C. sphenoides(4), C. symbiosum(2), C. xylanolyticum(4), C. xylanovorans(2).* *Eubacterium rectale*$*(2), E. hallii(3), E. ruminantium(2), E. cellulosolvens(3), E. contortum(3), E. eligens(4), E. ramulus(4), E. xylanophilum(3).* *Ruminococcus. gnavus*$*(2), R. hansenii*$*(0), R. luti(0), R. obeum(2), R. hydrogenotrophicus(3), R. lactaris(2), R. schinkii(2), R. torques(3).* *Others: see TableS1*
Bacteroides/ *Prevotella* group	*Bacteroides fragilis*$*(0), B. vulgatus*$(1), *B. uniformis*$*(2), B. eggerthii*$*(2), B. ovatus*$*(1), B. thetaiotaomicron*$*(0), B. caccae*$*(1), B. acidifaciens(2), B. stercoris(0), B. plebeius(0), B. splanchnicus(5), B. salyersiae(0), B. nordii(0), B. plebeius(0), B. coprocola(0), B. massiliensis(1), B. intestinalis(2), B. finegoldii(0), B. dorei(2), Parabacteroides distasomis(1).* *Prevotella albensis*$*(4), P. bivia(5), P. Bryantii(4), P. buccalis(5), P. denticola(5), P. disiens(5), P. enoeca(5), P. heparinolytica(0), P. intermedia(4), P. melaninogenica(5), P. multiformis(4), P. nigrescens(5), P. oris(6), P. oulorum(5), P. pallens(5), P. salivae(5), P. tannerae(1), P. veroralis(5), P. zoogleoformans(0).*
Bifidobacterium genus	*Bifidobacterium adolescentis*$*(0), B. longum XX bv infantis*$*(0), B. animalis(0), B. breve*$*(1), B. choerinum(0), B. gallicum(0), B. thermacidophilum(0), B. boum(0), B. merycicum(0), B. ruminantium(0), B. angulatum(0), B. pseudocatenulatum(0), B. dentium(0), B. gallinarum(0), B. saeculare(0), B. pullorum(0), B. longum(0), B. pseudolongum(0), B. indicum(1), B. bifidum(1), B. catenulatum(2), B. asteroides(1), B. coryneforme(0), B. cuniculi (1), B. minimum(0), B. scardovii(0), B. psychraerophilum(2), B. subtile(0).* *Others: see TableS1*
Lactobacillus/ Leuconostoc/ Pediococcus group	*Lactobacillus acidophilus*$*(0), L. casei*$*(0), L.paracasei*$*(0), L. delbrueckii*$*(0), L. fermentum*$*(0), L. helveticus*$*(0), L. johnsonii*$*(0), L. plantarum*$*(0), L. rhamnosus*$*(0), L. crispatus*$*(0), L. salivarius*$*(0), L. gasseri*$*(0), L. mucosae*$*(0), L. acetotolerans(0), L. acidifarinae(0), L. acidipiscis(0), L. agilis(0), L. alimentarius(0), L. amylophilus(0), L. amylovorus(0), L. antri(0), L. aviarius(0), L. bifermentans(0), L. brevis(0), L. buchneri(0), L. coleohominis(0), L. collinoides(0), L. concavus(0), L. coryniformis(0), L. curvatus(0), L. durianis(0), L. equi(0), L. farciminis(0), L. fornicalis(0), L. fructivorans(0), L. frumenti(0), L. fuchuensis(0), L. gallinarum(2), L. gastricus(0), L. graminis(0), L. hammesii(0), L. harbinensis(0), L. hilgardii(0), L. homohiochii(1), L. ingluviei(0), L. intestinalis(0), L. jensenii(0), L. kalixensis(0), L. keferi(0), L. kefiranofaciens(0), L. kimchii(0), L. kitasatonis(0), L. kunkeei(0), L. lindneri(2), L. malefermentans(0), L. mali(0), L. manihotivorans(0), L. mindensis(0), L. murinus(0), L. pontis(0) L. oligofermentans(0), L. oris(0), L. panis(0), L. pantheris(0), L. parabrevis(0), L. parabuchneri(0), L. paracollinoides(0), L. parakefiri(0), L. paralimentarius(0), L. paraplantarum(0), L. pentosus(0), L. perolens(0), , L. rennini(0), L. reuteri(0), L. pseudomesenteroides(0), L. rossii(0), L. ruminis(0), L. sakei(0),L. saerimneri(0), L. salivarius(0), L. sanfranciscensis(2), L. vini(0), L. satsumensis(0), L. sharpeae(0), L. siligionis(0), L. sobrius(0), L. spicheri(0), L. suebicus(0), L. vaccinostercus(0), L. vaginalis(1), L. versmoldensis(0), L. zeae(0).*

	Leuconostoc mesenteroides$*(0), Ln pseudomesenteroides*$*(1), Ln durionis(1), Ln fructosum(1), Ln ficulneum(1), Ln gelidum(1), Ln gasicomitatum(1), Ln inhae(1), Ln gelidum(1), L. kimchii(1), Ln lactis(0), Ln pseudoficulneum(1), L. fallax(1). Pediococcus inopinatus(0), P. parvulus(0), P. celliocola(0), P. acidilactici(0), P. pentosaceus(0), P. claussenii(0), P. stilesii(0), P. dextrinicus(0).*

*: Target species were obtained by using Probe Match program (Ribosomal Database Project II) (Maidak *et al.*, 2001) by checking each probe and primers with the following data set options: Strain: type, Source: isolates, Size: ≥ 1200 and < 1200 nt, Quality: good. Homology of the TaqMan probe was absolute as previously described (Holland *et al.*, 1991). OligoCheck v1 (http://www.cf.ac.uk/biosi/research/biosoft) was used to assist in primer design and to confirm the specificity of primers and probes. The maximum mismatch number determined by Oligocheck for the Type-strain sequences is shown in brackets. The positions of mismatches are showed in the supplementary file (Table S1).

$ **:** Species tested as control in real-time qPCR (cf. Table 3).

1 Table 3. Specificity of oligonucleotide primers and probes in real-time PCR assessed using pure bacterial culture DNA.
2 [a] + : positive, -: negative. * Abbreviations ATCC, DSM, VPI and NCTC referred to the strain names in commercial collections.
3 UEPSD and CNRZ corresponded to two INRA collections in Jouy-en-Josas.
4

Strain	Origin*	PCR results with each primer set [a]								
		Bacteria	*C. leptum*	*C. coccoides*	*Bacteroides/ Prevotella*	*Bifido-bacterium*	*E. coli*	*S. salivarius*	*Lactobacillus/ Leuconostoc/ Pediococcus*	*Entero-coccus*
Clostridium leptum	ATCC 29065	+	+	-	-	-	-	-	-	-
Faecalibacterium prausnitzii	UEPSD L43	+	+	-	-	-	-	-	-	-
Ruminococus albus	UEPSD M30	+	+	-	-	-	-	-	-	-
Clostridium coccoides	ATCC 29236	+	-	+	-	-	-	-	-	-
Ruminococus gnavus	ATCC 29149	+	-	+	-	-	-	-	-	-
Ruminococus hansenii	DSM 20583^T	+	-	+	-	-	-	-	-	-
Eubacterium rectale	UEPSD A4	+	-	+	-	-	-	-	-	-
Bacteroides fragilis	ATCC43185	+	-	-	+	-	-	-	-	-
Bacteroides ovatus	ATCC 8483	+	-	-	+	-	-	-	-	-
Bacteroides thetaiotaomicron	ATCC 29148	+	-	-	+	-	-	-	-	-
Bacteroides uniformis	ATCC 8492	+	-	-	+	-	-	-	-	-
Bacteroides vulgatus	ATCC 8482	+	-	-	+	-	-	-	-	-
Bacteroides caccae	ATCC 43185	+	-	-	+	-	-	-	-	-
Bacteroides eggerthii	UEPSD L78	+	-	-	+	-	-	-	-	-
Prevotella oralis	DSM 20702^T	+	-	-	+	-	-	-	-	-
Prevotella buccae	DSM 20615	+	-	-	+	-	-	-	-	-
Prevotella albensis	DSM 11730^T	+	-	-	+	-	-	-	-	-
Bifidobacterium adolescentis	ATCC15703	+	-	-	-	+	-	-	-	-
Bifidobacterium breve	ATCC15700	+	-	-	-	+	-	-	-	-
Biffidobacterium infantis	ATCC 15697	+	-	-	-	+	-	-	-	-
Escherichia coli	UEPSD S123	+	-	-	-	-	+	-	-	-

Streptococcus salivarius	DSM 20067	+	-	-	-	-	-	+	-	-
Streptococcus thermophilus	DSM 20259	+	-	-	-	-	-	+	-	-
Streptococcus vestibularis	DSM 5636^{T}	+	-	-	-	-	-	+	-	-
Lactobacillus acidophilus	UEPSD R52	+	-	-	-	-	-	-	+	-
Lactobacillus casei	CNRZ	+	-	-	-	-	-	-	+	-
Lactobacillus paracasei	CNRZ	+	-	-	-	-	-	-	+	-
Lactobacillus delbrueckii	CNRZ	+	-	-	-	-	-	-	+	-
Lactobacillus fermentum	CNRZ	+	-	-	-	-	-	-	+	-
Lactobacillus johnsonii	CNRZ	+	-	-	-	-	-	-	+	-
Lactobacillus plantarum	CNRZ	+	-	-	-	-	-	-	+	-
Lactobacillus rhamnosus	UEPSD R11	+	-	-	-	-	-	-	+	-
Lactobacillus helveticus	CNRZ	+	-	-	-	-	-	-	+	-
Lactobacillus crispatus	DSM 20584^{T}	+	-	-	-	-	-	-	+	-
Lactobacillus salivarius	DSM 20555^{T}	+	-	-	-	-	-	-	+	-
Lactobacillus gasseri	DSM 20243^{T}	+	-	-	-	-	-	-	+	-
Lactobacillus mucosae	DSM 13345^{T}	+	-	-	-	-	-	-	+	-
Leuconostoc mesenteroides	CNRZ	+	-	-	-	-	-	-	+	-
Ln pseudomesenteroides	CNRZ	+	-	-	-	-	-	-	+	-
Enterococcus faecium	UEPSD L99	+	-	-	-	-	-	-	-	+
Enterococcus faecalis	UEPSD L98	+	-	-	-	-	-	-	-	+
Clostridium perfringens	ATCC 13124	+	-	-	-	-	-	-	-	-
Clostridium sordelii	VPI 9048	+	-	-	-	-	-	-	-	-
Atopobium parvulum	UEPSD B69	+	-	-	-	-	-	-	-	-
Atopobium vaginae	DSM 15829^{T}	+	-	-	-	-	-	-	-	-
Atopobium rimae	DSM 7090^{T}	+	-	-	-	-	-	-	-	-
Clostridium bifermentans	NCTC 506	+	-	-	-	-	-	-	-	-
Streptococcus gordonii	DSM 6777^{T}	+	-	-	-	-	-	-	-	-

1
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Table 4: Composition of human faecal microbiota compared to farm animal microbiota.
n represents the numbers of studied samples.
(a) All-bacteria results obtained by qPCR were expressed as the mean of the log10 value ± SEM.
(b) Results were expressed as the mean of the log10 value ± SEM of normalized data, calculated as the log no. of targeted bacteria minus the log of All-bacteria number.
The reference for the statistics is with human faecal samples. The non parametric Wilcoxon test was performed if the One-way ANOVA for the bacterial group was significant.
Data not sharing the same letter within a column are significantly different to the human population, at $P<0.05$.

		TaqMan detection					**SYBR-Green detection**			
	n	All-bacteria (a)	*C. leptum* group (b)	*C. coccoides* group (b)	*Bacteroides/ Prevotella* group (b)	*Bifidobacterium* genus (b)	*Lactobacillus/ Leuconostoc/ Pediococcus* group (b)	*E. coli* (b)	*S. salivarius* species (b)	*Enteroccocus* genus (b)
Human	21	11.5 ± 0.1	-0.7 ± 0.05 (A)	-1.3 ± 0.08 (A)	-1.5 ± 0.06 (A)	-2.4 ± 0.33 (A)	-3.9 ± 0.13 (A)	-3.8 ± 0.34 (A)	-3.1 ± 0.12 (A)	-5.0 ± 0.15
Horse	5	11.5 ± 0.1	-1.5 ± 0.06 (B)	-2.2 ± 0.18 (B)	-2.3 ± 0.04 (B)	-4.8 ± 0.13 (B)	-2.4 ± 0.82 (A)	-5.0 ± 0.03 (B)	-5.2 ± 0.20 (B)	Not detected
Cow	5	11.4 ± 0.1	-1.0 ± 0.03 (B)	-2.6 ± 0.03 (B)	-2.3 ± 0.01 (B)	-3.6 ± 0.37 (B)	-3.1 ± 0.06 (A)	-5.0 ± 0.31 (B)	-5.0 ± 0.04 (B)	Not detected
Goat	5	12.0 ± 0.1	-1.0 ± 0.07 (B)	-2.2 ± 0.11 (B)	-2.4 ± 0.19 (B)	-1.8 ± 0.26 (A)	-3.2 ± 0.78 (A)	-4.5 ± 0.48 (A)	-4.3 ± 0.43 (B)	Not detected
Rabbit	5	11.7 ± 0.1	-0.7 ± 0.03 (A)	-1.9 ± 0.03 (B)	-1.2 ± 0.09 (A)	-1.6 ± 0.07 (A)	-5.1 ± 0.59 (A)	Not detected	Not detected	Not detected
Sheep	5	11.9 ± 0.1	-1.0 ± 0.05 (B)	-2.7 ± 0.08 (B)	-2.4 ± 0.08 (B)	-4.2 ± 0.11 (B)	-5.3 ± 0.60 (A)	-4.1 ± 0.52 (A)	Not detected	Not detected
Pig	5	11.9 ± 0.1	-1.2 ± 0.11 (B)	-1.7 ± 0.35 (A)	-1.9 ± 0.17 (A)	-3.4 ± 0.69 (B)	-1.2 ± 0.54 (B)	-2.7 ± 0.06 (B)	Not detected	Not detected

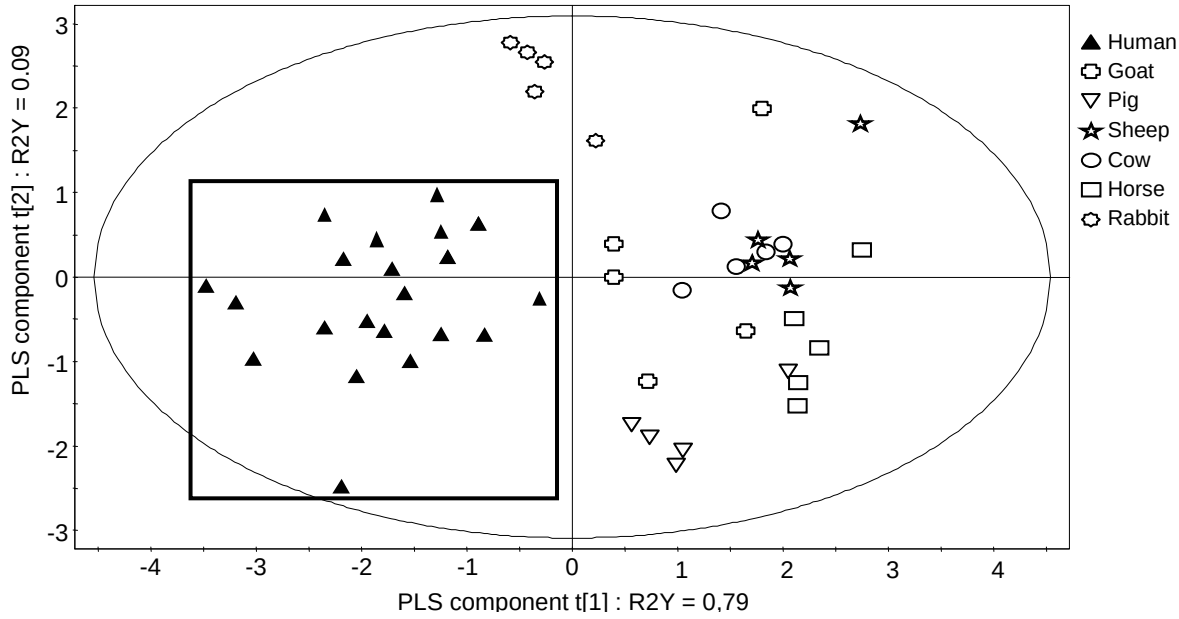
Figure legend.

Figure 1. PLS discrimination between microbiota of human and farm animals.

A. Relationship between faecal microbiota (variables *X*) and human or farm animals (variables *Y*) using PLS regression. The cross-validation led to two components represented here as t(1) and t(2). The corresponding PLS model explains 80.0 % of the variation of the *Y*-matrix. The 95 % probability region defined by the model is delimited by the ellipse. The human (▲) group (n=21) can be distinguished and is delimited by the black square. ◯: cow (n=5); □: horse (n=5); ▽: pig (n=5); ⯎: rabbit (n=5); ☆: sheep (n=5); ⯐ goat (n=5).

B. The window shows the *X*-loadings (*w**) of the *X* variables (faecal microbiota quantifications) and the *Y*-loadings (*c*) of the *Y* variables (human and animal groups), and thereby shows the correlation between *X* and *Y*. The *X* (black triangles) and *Y* (black circles) variables combine in the projections, and the *X* variables relate to the *Y* variables, as shown in the figure. The *C. coccoides* group, *S. salivarius* species and *Enterococcus* genus, significant for the discrimination of human and farm animals, and the Lactobacillus*/Leuconostoc/Pediococcus* group, characterizing the pig microbiota, are denoted by large black triangles (small black triangles represent less significant *X* variables).

A


PLS component t[2] : R2Y = 0.09
PLS component t[1] : R2Y = 0,79
Human
Goat
Pig
Sheep
Cow
Horse
Rabbit

B

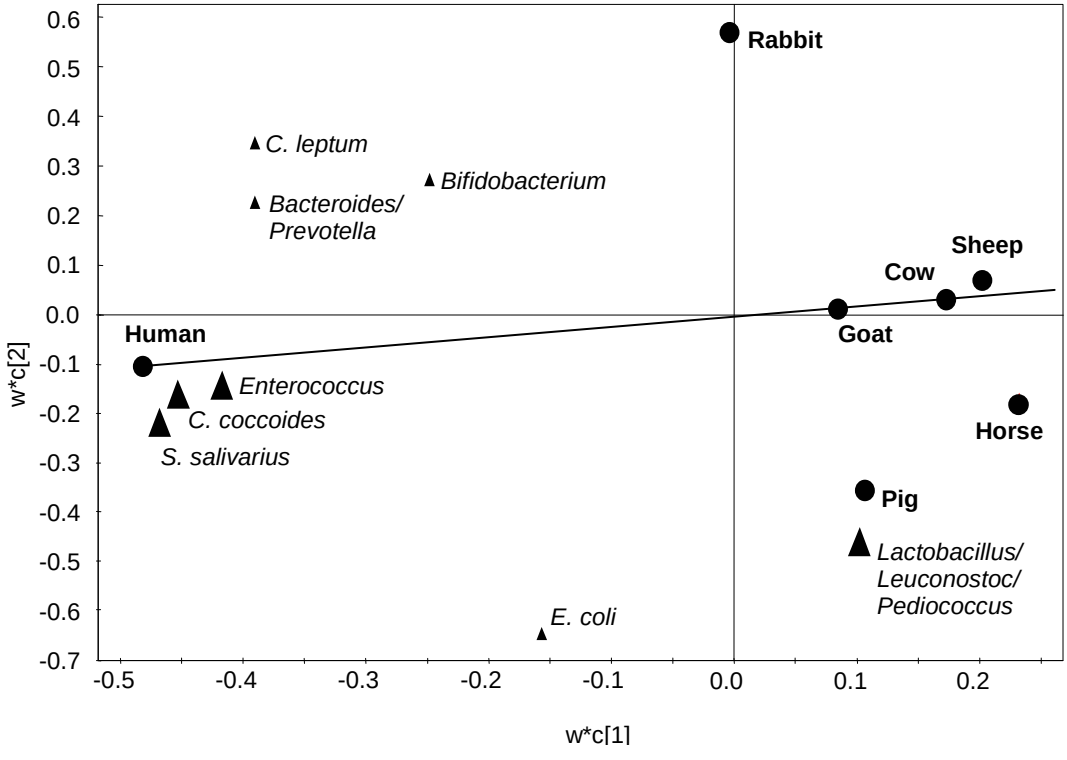

w*c[2]
w*c[1]
Rabbit
C. leptum
Bifidobacterium
Bacteroides/
Prevotella
Sheep
Cow
Goat
Human
Enterococcus
C. coccoides
S. salivarius
Horse
Pig
Lactobacillus/
Leuconostoc/
Pediococcus
E. coli

Figure 1.

Table S1: Sequence alignment of the species targeted by OligoCheck software showing sequence differences. Only those nucleotides that are different from the target sequence for each organism are shown.

[(1)]. As indicated, there were two exceptions to the rule of absolute homology for the TaqMan probes based on this silico assessment. The type strain *P. oralis* DSM20702^{T} and the strain *P. buccae* DSM20615 have one and two C/T mismatches, respectively, and are positive in PCR assays (Table 3).

PCR assays: positive: positive PCR, negative: negative PCR, nt: no tested

Clostridium leptum group

Species	EMBL Access Number	F_Clept09 primer 5′ 3′ CCTTCCGTGCCGSAGTTA	P_Clept01 probe 5′ 3′ CACAATAAGTAATCCACC	R_Clept08 primer 5′ 3′ GAATTAAACCACATACTCCACTGCTT	PCR assay
Clostridium					
C. leptum	AJ305238	--C----------	----------	----------	positive
C. methylpentosum	Y18181	---T------G-----	----------	----------	nt
C. sporosphaeroides	M59116	-N----------G-----	----------	----------	nt
F. prausnitzii	AJ413954	-----A----------	----------	----------	positive
Ruminococcus					
R. callidus	L76596	----------	----------	----------	nt
R. flavefaciens	L76603	----------	----------	----------	nt
R. albus	L76598	----------	----------	----------	positive
R. bromii	L76600	----------G-----	----------	----------	nt
Eubacterium					
Eub. siraeum	L34625	----------	----------	----------	nt
Other genera					
Ac. elongatum	AY487928	----------	----------	----------	nt
Anaerotruncus colihominis	AJ315980	----------	----------	----------	nt
An. pentosovorans	X97852	-T----------	----------	----------	nt
An. Agile	X98011	-TC----------	----------	----------	nt

Bifidobacterium genus

Species	EMBL Access Number	F_Bifid 09c primer 5′ 3′ CGGGTGAGTAATGCGTGACC	P_Bifid probe 5′ 3′ CTCCTGGAAACGGGTG	R_Bifid 06 primer 5′ 3′ TGATAGGACGCGACCCCA	PCR assay
Bifidobacterium					
B. infantis	D86184	--------------------	----------------	----------------	positive
B. animalis	X89513	--------------------	----------------	----------------	nt
B. choerinum	D86186	--------------------	----------------	----------------	nt
B. gallicum	D86189	--------------------	----------------	----------------	nt
B. thermacidophilum	AY148470	--------------------	----------------	----------------	nt
B. boum	D86190	--------------------	----------------	----------------	nt
B. merycicum	D86192	--------------------	----------------	----------------	nt
B. ruminantium	D86197	--------------------	----------------	----------------	nt
B. angulatum	D86182	--------------------	----------------	----------------	nt
B. pseudocatenulatum	D86187	--------------------	----------------	----------------	nt
B. dentium	D86183	--------------------	----------------	----------------	nt
B. gallinarum	D86191	--------------------	----------------	----------------	nt
B. saeculare	D89328	--------------------	----------------	----------------	nt
B. pullorum	D86196	--------------------	----------------	----------------	nt
B. longum	M58739	--------------------	----------------	----------------	nt
B. pseudolongum	M58742	--------------------	----------------	---------N--------	nt
B. indicum	M58737	-----N---------------	----------------	---------N--------	nt
B. breve	M58731	----N----------------	----------------	----------------	positive
B. bifidum	M38018	--------------------	----------------	---------N--------	nt
B. adolescentis	M58729	--------------------	----------------	----------------	positive
B. catenulatum	M58732	---NN----------------	----------------	---------N--------	nt
B. asteroides	M58730	--------------------	----------------	----------------N	nt
B. coryneforme	M58733	--------------------	----------------	----------------	nt
B. cuniculi	M58734	--------------------	----------------	----------------	nt
B. minimum	M58741	--------------------	----------------	----------------	nt
B. scardovii	AJ307005	--------------------	----------------	----------------	nt
B. psychraerophilum	AY174108	------------A------T	----------------	----------------	nt
B. subtile	D89378	--------------------	----------------	----------------	nt
Others					
Brevundimonas diminuta	M59064	-----------CA----G-A	----------------	A---A------GG---GC	nt
Mycoplana bullata	D12785	-----------CA----G-A	----------------	A---A------GG---GC	nt
Parascardovia denticolens	D89331	-------------------T	----------------	----------------	nt
Scardoviaino pinata	AB029087	-------------------T	----------------	----------------	nt

Clostridium coccoides group

Species	EMBL Access Number	F_Ccocc07 primer 5′ 3′ GACGCCGCGTGAAGGA	P_Erec482 probe 5′ 3′ CGGTACCTGACTAAGAAG	R_Ccocc14 primer 5′ 3′ AGCCCCAGCCTTTCACATC	PCR assay
Clostridium					
C. coccoides	M59090	----------	----------N-----	--N----------	positive
C. aerotolerans	X76163	----------GT--	----------	--------G-------TC-	nt
C. algidixylanolyticum	AF092549	----------CT--	----------	------G-G-------TC-	nt
C. aminophilum	L04165	----------GC--	----------	------G-G----------	nt
C. aminovalericum	X73436	----------GT--	----------	------G--NN-----AC-	nt
C. amygdalinum	AY353957	----------CT--	----------	------G-G-------TC-	nt
C. bolteae	AJ508452	----------GT--	----------	-----TG-GT------T--	nt
C. celerecrescens	X71848	----------GT--	----------	------G-G-------TC-	nt
C. clostridioforme	M59089	----------GT--	----------	------G-G----------	nt
C. hathewayi	AJ311620	----------GT--	----------	------G-G-------T--	nt
C. herbivorans	L34418	----------GT--	----------	--A---------C------	nt
C. hylemonae	AB023973	----------	----------	------G-G----------	nt
C. indolis	Y18184	----------GT--	----------	------G-G-------TC-	nt
C. jejuense	AY494606	----------GT--	----------	------G-G----------	nt
C. lentocellum	X71851	----------	----------	------TAAA-------T-	nt
C. nexile	X73443	----------GC--	----------	------G-G----------	nt
C. oroticum	M59109	----------	----------	---NNNNNNG---------	nt
C. populeti	X71853	----------GT--	----------	------G-G----------	nt
C. proteoclasticum	U37378	----------GT--	----------	------G-G----------	nt
C. saccharolyticum	Y18185	----------GT--	----------	------G-G-------TC-	nt
C. scindens	AF262238	----------	----------	------G-G----------	nt
C. sphenoides	X73449	----------GT--	----------	------G-G-------TC-	nt
C. symbiosum	M59112	----------GT--	----------	------G-G----------	nt
C. xylanolyticum	X71855	----------GT--	----------	------G-G-------TC-	nt
C. xylanovorans	AF116920	----------GT--	----------	------G-G----------	nt
Eubacterium					
E.hallii	L34621	A----------GT--	----------	------G----------	nt
E.ruminantium	AB008552	----------GT--	----------	------G--A---------	nt
E. cellulosolvens	X71860	----------GT--	----------	------A-GT------T--	nt
E. contortum	L34615	----------	----------	------G-GT---------	nt
E. eligens	L34420	----------GT--	----------	------N-AT------T--	nt
E. ramulus	L34623	----------GC--	----------	---N--G-G-------A--	nt
E. rectale	L34627	----------GC--	----------	------G-G----------	positive
E. xylanophilum	L34628	----------GT--	----------	------GN-T---------	nt
Ruminococcus					
R.gnavus	L76597	----------GC--	----------	N-N----------	positive
R.hansenii	M59114	----------	----------	----------	positive
R.hydrogenotrophicus	X95624	----------	----------	------TA--------A--	nt
R.lactaris	L76602	----------GC--	----------	------G-G----------	nt
R.luti	AJ133124	----------	----------	----------	nt
R.obeum	L76601	----------	----------	------C---------A--	nt
R.schinkii	X94965	----------	----------	------T---------A--	nt
R.torques	D14137	----------GC--	----------	------G-GT---------	nt
Others					
Coprococcus catus	AB038359	----------	----------	------G--NN-----T--	nt
Coprococcus eutactus	D14148	G—C---------GT--	----------	------G--A------T--	nt
Desulfotomaculum guttoideum	Y11568	----------GT--	----------	------G-G-------TC-	nt
Dorea formicigenerans	L34619	----------	----------	-----AT---------T--	nt
Dorea longicatena	AJ132842	----------	----------	-----GG-CT---------	nt
Hespellia porcina	AF445239	----------	----------	------G-G----------	nt
Hespellia stercorisuis	AF445264	----------	----------	------G-G----------	nt
Lachnobacterium bovis	AF298663	----------AC--	----------	------G-G----------	nt
Lachnospira pectinoschiza	L14675	----------GT--	----------	------G-G----------	nt
Pseudobutyrivibrio ruminis	X95893	----------GC--	----------	------G-G----------	nt
Roseburia cecicola	L14676	----------GC--	----------	------G-N----------	nt
Roseburia intestinalis	AJ312385	----------GC--	----------	------G-G----------	nt
Syntrophococcus sucromutans	Y18191	----------GT--	----------	------G-G----------	nt
Acetitomaculum ruminis	M59083	A-T---------AG--	----------	G-----N-GA---------	nt
Anaerostipes caccae	AJ270487	----------GT--	----------	------G-G-------T--	nt
Catonella morbi	X87151	----------GT--	----------	------CTG---C---TCT	nt

Bacteroides / *Prevotella* group

Species	EMBL Access Number	F_Bacter 11 primer 5′ 3′ CCTWCGATGGATAGGGGTT	P_Bact303 probe 5′ 3′ AAGGTCCCCCACATTG	R_Bacter 08 primer 5′ 3′ CACGCTACTTGGCTGGTTCAG	PCR assay
Bacteroides					
B. acidifaciens	AB021164	A-A----------	----------	----------	positive
B. caccae	X83951	A----------	----------	----------	positive
B. coprocola	AB200224	----------	----------	----------	nt
B. dorei	AB242142	T-A----------	----------	----------	nt
B. eggerthii	L16485	T-A----------	----------	----------	positive
B. finegoldii	AB222699	----------	----------	----------	nt
B. fragilis	X83935	----------	----------	----------	positive
B. intestinalis	AB214328	A-A----------	----------	----------	nt
B. massiliensis	AY126616	T----------	----------	----------	nt
B. nordii	AY608697	----------	----------	----------	nt
B. ovatus	X83952	A----------	----------	----------	positive
B. plebeius	AB200217	----------	----------	----------	nt
B. salyersiae	AY608696	----------	----------	----------	nt
B. splanchnicus	L16496	A-G-T---A-G--------	----------	-----A----------	nt
B. stercoris	X83953	----------	----------	----------	nt
B. thetaiotaomicron	L16489	----------	----------	----------	positive
B. uniformis	L16486	---N--------N------	----------	-----N----------	positive
B. vulgatus	M58762	T----------	----------	----------	positive
Parab. distasonis	M86695	--G----------	----------	-----A----------	nt
Prevotella					
Prevotella albensis	AJ011683	--G-----CAG--------	----------	----------	positive
P. bivia	L16475	G-A-----CAG--------	----------	----------	nt
P. bryantii	AJ006457	--G-----CAG--------	----------	----------	nt
P. buccae ***(1)***	L16478	--------C-G--------	----C--------C--	----------	positive
P. buccalis	L16476	G-G-----CAG--------	----------	----------	nt
P. denticola	L16467	G-G-----CAG--------	----------	--N----------	nt
P. disiens	L16483	G-A-----CAG--------	----------	----------	nt
P. enoeca	AJ005635	G-A-----CAG--------	----------	----------	nt
P. heparinolytica	L16487	----------	----------	----------	nt
P. intermedia	X73965	G-------CAG--------	----------	----------	nt
P. melaninogenica	L16469	G-A-----CAG--------	----------	----------	nt
P. multiformis	AB182483	G-------CAG--------	----------	----------	nt
P. nigrescens	L16471	G-G-----CAG--------	----------	----------	nt
P. oralis ***(1)***	L16480	G-A-----C-G--------	----------C--	----------	positive
P. oris	L16474	G-GN----CAG--------	----------	----------	nt
P. oulorum	L16472	G-A-----CAG--------	----------	----------	nt
P. pallens	Y13105	G-N-----CAG--------	----------	----------	nt
P. salivae	AB108826	G-A-----CAG--------	----------	----------	nt
P. tannerae	AJ005634	----------G--------	----------	----------	nt
P. veroralis	L16473	G-G-----CAG--------	----------	----------	nt
P. zoogleoformans	L16488	----------	----------	----------	nt

Lactobacillus / Leuconostoc / Pediococcus group

Species	EMBL Access number	F_lacto05 primer 5′ 3′ AGCAGTAGGGAATCTTCCA	R_LactoO4 Primer 5′ 3′ CGCCACTGGTGTTCYTCCATATA	PCR assay
Lactobacillus				
L. acetotolerans	M58801	----------	----------	nt
L. acidifarinae	AJ632158	----------	----------	nt
L. acidipiscis	AB023836	----------	----------	nt
L. acidophilus	m58802	----N-----	----------	positive
L. agilis	M58803	----------	----------	nt
L. algidus	AB033209	----------	----------A-------	nt
L. alimentarius	M58804	----------	----------	nt
L. amylophilus	M58806	----------	----------	nt
L. amylovorus	m58805	----------	----------	nt
L. animalis	M58807	----------N-------N	----------	nt
L. antri	AY253659	----------	----------	nt
L. aviarius	M58808	----------	----------	nt
L. bifermentans	M58809	----------	----------	nt
L. brevis	ab070611	----------	----------	nt
L. buchneri	M58811	----------	----------	nt
L. casei	D16548	----------	----------	positive
L. coleohominis	AM113776	----------	----------	nt
L. collinoides	AB005893	----------	----------	nt
L. concavus	AY683322	----------	----------	nt
L. coryniformis	AJ575741	----------	----------	nt
L. crispatus	y17362	----------	----------	positive
L. curvatus	AJ270951	----------	----------	nt
L. delbrueckii	x52654	----------	----------	positive
L. durianis	AJ315640	----------	----------	nt
L. equi	AB048833	----------	----------	nt
L. farciminis	M58817	----------	----------	nt
L. fermentum	af522394	----------	----------	positive
L. fornicalis	Y18654	----------	----------	nt
L. fructivorans	m58818	----------	----------	nt
L. frumenti	AJ250074	----------	----------	nt
L. fuchuensis	AB063479	----------	----------	nt
L. gallinarum	AJ242968	-A----------C	----------	nt
L. gasseri	m58820	----------	----------	positive
L. gastricus	AY253658	----------	----------	nt
L. graminis	AM113778	----------	----------	nt
L. hammesii	AJ632219	----------	----------	nt
L. harbinensis	AB196123	----------	----------	nt
L. helveticus	ay369116	----------	----------	positive
L. hilgardii	M58821	----------	----------	nf
L. homohiochii	AM113780	----------	----------A-------	nf
L. ingluviei	AF317702	----------	----------	nf
L. intestinalis	aj306299	----------	----------	nf
L. jensenii	AF243176	----------	----------	nf
L. johnsonii	aj002515	----------	----------	positive
L. kalixensis	AY253657	----------	----------	nt
L. keferi	AJ621553	----------	----------	nt
L. kefiranofaciens	AM113781	----------	----------A-------	nt
L. kimchii	AF183558	----------	----------	nt
L. kitasatonis	AB107638	----------	----------	nt
L. kunkeei	Y11374	----------	----------	nt
L. lindneri	X95421	----------	----------C-T------	nt
L. malefermentans	AM113783	----------	----------	nt
L. mali	M58824	----------	----------	nt
L. manihotivorans	AF000162	----------	----------	nt
L. mésenteroides	m23035	T----------	----------	nt
L. mindensis	AJ313530	----------	----------	nt
L. mucosae	AF126738	----------	----------	positive
L. murinus	M58826	----------	----------	nt
L. oligofermentans	AY733084	----------	----------	nt
L. oris	X94229	----------	----------	nt
L. panis	X94230	----------	----------	nt
L. pantheris	AF413523	----------	----------	nt
L. parabrevis	AM158249	----------	----------	nt
L. parabuchneri	AY026751	----------	----------	nt
L. paracasei	d79212	----------	----------	positive
L. paracollinoides	AJ786665	----------	----------	nt
L. parakefiri	AY026750	----------	----------	nt
L. paralimentarius	AJ417500	----------	----------	nf
L. paraplantarum	AJ306297	----------	----------	nt
L. pentosus	D79211	----------	----------	nt

L. perolens	Y19167	----------	----------	nt
L. plantarum	D79210	----------	----------	positive
L. pontis	AJ422032	----------	----------	nt
L. pseudomesenteoides	ab023237	----------	----------	nt
L. rennini	AJ576007	----------	----------	nt
L. reuteri	l23507	----------	----------	nt
L. rhamnosus	m58815	----------	----------	positive
L. rossii	AJ564009	----------	----------	nt
L. ruminis	m58828	----------	----------	nt
L. saerimneri	AY255802	----------	----------	nt
L. sakei	ay204897	----------	----------	nt
L. salivarius	af089108	----------	----------	positive
L. sanfranciscensis	X76327	----------	----------C-T------	nt
L. satsumensis	AB154519	----------	----------	nt
L. sharpeae	m58831	----------	----------	nt
L. siligionis	DQ168027	----------	----------	nt
L. sobrius	AY700063	----------	----------	nt
L. spicheri	AJ534844	----------	----------	nt
L. suebicus	AJ306403	----------	----------	nt
L. vaccinostercus	AM113786	----------	----------	nt
L. vaginalis	x61136	----------	----------N-	nt
L. versmoldensis	AJ496791	----------	----------	nt
L. vini	AJ576009	----------	----------	nt
L. zeae	D86516	----------	----------	nt
Leuconostoc				
Leuco mesenteroides	m23035	----------	----------	positive
Leuco pseudomesenteroides	ab023237	T----------	----------	positive
Pediococcus				
P.inopinatus	AJ271383	----------	----------	nt
P.parvulus	D88528	----------	----------	nt
P.cellicola	AY956788	----------	----------	nt
P.acidilactici	M58833	----------	----------	nt
P.pentosaceus	M58834	----------	----------	nt
P.claussenii	AJ621555	----------	----------	nt
P.stilesii	AJ973157	----------	----------	nt
P.dextrinicus	D87679	----------	----------	nt
Enterococcus				
E.aquimarinus	AJ877015	----------GG	----------	nt
E.asini	Y11621	----------GG	----------	nt
E.avium	AF133535	----------GC	----------	nt
E.caccae	AY943820	----------GC	----------	nt
E.canintestini	AJ888906	----------GC	----------	nt
E.casseliflavus	Y18161	----------GC	----------	nt
E.cecorum	AF061009	----------GC	----------	nt
E.devriesei	AJ891167	----------GC	----------	nt
E.dispar	AF061007	----------GC	----------	nt
E.durans	AJ420801	----------GC	----------	nt
E.faecalis	af515223	----------GC	----------C------	negative
E.faecium	AJ276355	----------GC	----------	negative
E.gilvus	AY033814	----------GC	----------	nt
E.hermanniensis	AY396047	----------GC	----------	nt
E.hirae	AJ276356	----------GC	----------	nt
E.italicus	AJ582753	----------GC	----------	nt
E.malodoratus	AF061012	----------GC	----------	nt
E.moraviensis	AF286831	----------GC	----------	nt
E.mundtii	AF061013	----------GC	----------	nt
E.pallens	AY033815	----------GC	----------	nt
E.phoeniculicola	AY028437	----------GC	----------	nt
E.pseudoavium	AF061002	----------GC	----------	nt
E.raffinosus	Y18296	----------GC	----------	nt
E.ratti	AF326472	----------GC	----------	nt
E.silesiacus	AM039966	----------GC	----------	nt
E.sulfureus	X55133	----------GC	----------	nt
E.termitis	AM039968	----------GC	----------	nt
E.villorum	AF335596	----------GC	----------	nt
Others				
Lactoccocus lactis	M58836	----------GC	------C-------C--------	nt
Streptococcus thermophilus	x68418	----------GC	------C---------C------	negative