Inter-Species Transplantation of Gut Microbiota from Human to Pigs

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Abstract:

Direct research on gut microbiota for understanding its role as 'an important organ' in human individuals is difficult owing to its vast diversity and host specificity as well as ethical concerns. Transplantation of human gut microbiota into surrogate hosts can significantly facilitate the research of human gut ecology, metabolism and immunity but rodents-based model provides results with low relevance to humans. A new human flora-associated (HFA) piglet model was hereby established taking advantage of the high similarity between pigs and humans with respect to the anatomy, physiology and metabolism of the digestive system. Piglets were delivered via cesarean section into a SPF-level barrier system and were inoculated orally with a whole fecal suspension from one healthy 10-year-old boy. The establishment and composition of the intestinal microbiota of the HFA piglets were analyzed and compared with that of the human donor using enterobacterial repetitive intergenic consensus sequence-PCR fingerprinting-based community DNA hybridization, group-specific PCR-temperature gradient gel electrophoresis and real-time PCR. Molecular profiling demonstrated that transplantation of gut microbiota from a human to germfree piglets produced a donor-like microbial community with minimal individual variation. And the microbial succession with aging of those ex-germfree piglets was also similar to that observed in humans. This HFA model provides a significantly improved system for research on gut ecology in human metabolism, nutrition and drug discovery.

Article:

INTRODUCTION

Human beings are 'superorganisms' that are colonized by 10^{14} total cells of more than 1000 species of bacteria, particularly in the gastrointestinal tract (Hooper and Gordon, 2001; Nicholson *et al.*, 2005). Each human subject has a unique gut microbiota, which is essential to host immunology, nutrition and pathogenesis. Genetically homogenous animals can have different metabolisms if they have structurally different gut microbiota, which in turn determines their individualized drug responses (Nicholson *et al.*, 2005; Clayton *et al.*, 2006). Structural shifts in gut microbiota have been suggested to play a role in the development of many non-infectious diseases such as obesity, diabetes and even autism in children (Backhed *et al.*, 2004; Kelly *et al.*, 2004; Parracho *et al.*, 2005; Ley *et al.*, 2006; Turnbaugh *et al.*, 2006).

The study of gut microbiota in diseased and healthy human subjects has been challenging owing to individual variation as well as ethical concerns. Transplantation of human gut microbiota into surrogate hosts, such as mice or rats, permits the generation of a human flora-associated (HFA) animal model with an established human-derived gut microbiota in place of its indigenous microbial ecosystem. Such HFA animal model has significantly facilitated progress in researches of human gut ecology, metabolism and immunity (Hazenberg *et al.*, 1981; Roland *et al.*, 1996; Oozeer *et al.*, 2002; Gerard *et al.*, 2004; Wilcks *et al.*, 2004). However, owing to the significant differences in basic anatomy and physiology of rodents to human, key members of human gut microbiota such as bifidobacteria do not colonize the rodent gut; and rodents do not develop a full repertoire of immune responses unless some rodent-specific gut bacteria are reintroduced back into the gut (Raibaud *et al.*, 1980; Imaoka *et al.*, 2004). Thus, results obtained from these mice/rat models often have low relevance to human beings.

The pig is an attractive non-primate animal model with a digestive tract similar in both physiology and anatomy to that of humans. Pigs eat an omnivorous diet and the developmental period is also similar to that of the human, especially during infancy (Garthoff *et al.*, 2002). In addition, swine are particularly susceptible to nutrient and metabolism modulation due to rapid growth.

The aim of the present study was to investigate the feasibility of transplantation of human gastrointestinal microbiota to the piglet intestine to establish a new HFA piglet model for gut ecology, nutrition and metabolism researches.

MATERIALS AND METHODS
Animals
Experimental piglets were of Meishan origin (native stock of China) and were delivered by cesarean section under germfree conditions. Neonatal piglets, one per cage, were housed in a temperature- and humidity-controlled SPF barrier system. Piglets were maintained at 37°C for the first 7 days, and then the temperature was gradually decreased to 30°C for the remainder of the experiment. Lights were on a 12-h light/dark cycle. Postnatal piglets were fed sterilized artificial milk formula specifically tailored for infant piglets (Anyou, Huaian, China). All piglets were weaned from 19 to 25 days of age. Commercial infant cereal (Nestlé Shuangcheng Ltd, Shuangcheng, China) was gradually introduced to the piglets to replace the milk formula. Twenty-eight piglets in three replicate trials (three litters; 7–12 piglets per litter) were used in the experiment.

Human whole fecal flora inoculation
Human fecal flora was obtained from one healthy boy (10-year old) who did not have diarrhea or other digestive disorders and had not received medication for at least 6 months before the study. Freshly passed stool was diluted 20-fold and homogenized in sterile pre-reduced 0.1 M potassium phosphate buffer (pH 7.2) containing 10% glycerol (vol/vol). The supernatant was then dispensed to cryotubes and immediately transferred to an environment with the temperature of -70°C. The human fecal suspension was administered orally by gavage to the newborn germfree piglets 12 h after their birth. The dosage was 1 ml/piglet once daily in the first 3 days, and then once every other day until 10 days of age.

Extraction of total genomic DNA

Fecal samples were prepared and total DNA was extracted as described previously (Wang *et al.*, 1996; Wei *et al.*, 2004). The DNA concentration was estimated by DyNA quantTM 200 (Amersham Pharmacia Biotech, San Francisco, CA, USA) and its integrity was examined by electrophoresis in 1% (wt/vol) agarose gels.

ERIC-PCR fingerprinting

Enterobacterial repetitive intergenic consensus sequence-PCR (ERIC-PCR) fingerprinting was used to monitor and characterize the structure of microbiota in the gastrointestinal tracts of the piglets. PCR was performed in a 25-μl reaction containing 80 ng of fecal genomic DNA, 200 μM (each) deoxynucleoside triphosphates, 2.5 U Taq DNA polymerase (Promega, Madison, WI, USA), 1 ×reaction buffer, 2 mM $MgCl_2$, and 0.4 μM of each primer (E1 and E2; Hulton *et al.*, 1991; Versalovic *et al.*, 1991). The amplification conditions were as follows: 7 min at 95°C, 30 cycles consisting of 30 s at 95°C, 1 min at 52°C, 8 min at 65°C and a final cycle of 16 min at 65°C (Versalovic *et al.*, 1991). The PCR products (400 ng) were separated by electrophoresis on a 1% (wt/vol) agarose gel containing ethidium bromide (0.25 μg/ml).

ERIC-PCR fingerprints were further analyzed using the UVI band/map software (UVItec, Cambridge, UK). This program clusters all the patterns by the unweighted pair-group method with arithmetic averages. The resulting dendrogram shows similar relationships between the profiles.

DNA fingerprinting-based community DNA hybridization

The ERIC-PCR products of all the samples were separated in agarose gels and then transferred onto Supercharge nylon membranes using a Vacu-Blot system. ERIC-PCR products of the human donor were DIG-labeled for use as the community probes (DIG-DNA label and detect kit; Roche Diagnostics, Mannheim, Germany), and hybridized with the ERIC-PCR profiles on nylon membranes. Community DNA hybridization was performed according to the method of Wei *et al.* (2004).

Group-specific PCR-TGGE

Bifidobacteria group-specific PCR that targets a partial 16S rRNA gene has been described by Satokari *et al.* (2001). PCR products were loaded onto 8% (wt/vol) polyacrylamide gels containing 8 M urea and 20% (vol/vol) formamide for separation. The thermal gradient was from 46°C to 54°C according to the results of prior perpendicular electrophoresis. Electrophoresis was performed in 1 ×TAE (Tris–acetate–EDTA (ethylenediaminetetraacetic acid)) buffer at a fixed voltage of 150 V for 3 h. TGGE gels were stained with 0.2% (wt/vol) $AgNO_3$ and developed according to the manufacturer's instructions. *Bacteroides* group-specific PCR-temperature gradient gel electrophoresis (PCR-TGGE) analysis of fecal samples was carried out using the Bfr-F and Bfr-GC-R primer pairs, as described previously (Pang *et al.*, 2005).

Cloning and sequencing analysis

Separate libraries of bifidobacteria group-specific and *Bacteroides* group-specific amplicons were constructed for the fecal sample of the donor. Group-specific PCR products were excised from a 0.8% (wt/vol) agarose gel, then purified and cloned using the pGEM-T easy vector

system (Promega, Madison, WI, USA). White clones were randomly selected and sequenced. Sequence analysis and homology searches of sequences in the GenBank database were performed using the BLAST algorithm (blastn). Inserted fragments of all sequenced amplicons were analyzed individually by TGGE and aligned with the original TGGE profile of the donor to identify the organism represented by each band.

Enumeration of bifidobacteria and Bacteroides spp using real-time PCR

Plasmid DNA standards for real-time PCR were prepared according to Bartosch *et al.* (2004). One positive clone was selected from each of the libraries specific for bifidobacteria and *Bacteroides* species. Plasmid DNA was extracted and the DNA concentration was determined. Plasmid DNA was serially diluted to generate a standard curve. The real-time PCR mixture (25 μl) was similar to that used to specifically amplify bifidobacteria species and *Bacteroides* species except for the addition of a 1:50 000 dilution of SYBR Green I. The amplification program was also the same as that described above for bifidobacteria and *Bacteroides* group-specific PCR. Fluorescence was detected during the last step of each cycle. After amplification, a melting curve was obtained using a slow temperature gradient from 60°C to 94°C (0.2°C increments with a hold time of 1 s), with continuous fluorescence collection. For each measurement, fluorescent signals were determined from three serial dilutions of target DNA, and the average value was compared to a standard curve generated in the same experiment.

Nucleotide sequence accession

Sequences of representative clones from this study were deposited in the GenBank database (accession no. AY756150 to AY756160; AY987029 to AY987034).

RESULTS AND DISCUSSION
Here we report the successful development of a HFA piglet model. The establishment of a human-donor like gut microbiota in ex-germfree piglets is the key for the success of this animal model.

Molecular techniques that do not require isolation of bacterial strains, such as ERIC-PCR fingerprinting, TGGE/denaturing gradient gel electrophoresis (DGGE) analysis, 16S rRNA gene clone library profiling and real-time PCR, were utilized to analyze the microbiota in the gut of the HFA piglets. Previous studies on the microbiota composition of HFA animals were based on traditional culture methods (Raibaud *et al.*, 1980; Hazenberg *et al.*, 1981; Mallett *et al.*, 1987; Hirayama, 1999), which underestimate the abundance of the bacterial populations and thus provide an incomplete picture of the composition of the microflora. Use of the above-mentioned molecular methods will yield more detailed information about the microbiota and will provide a more comprehensive comparison of the microbiota in the HFA animals and the human donor.

The development of microbiota in HFA piglet gut was monitored dynamically using ERIC-PCR fingerprinting. As one form of long primer randomly amplified polymorphic DNA (LP-RAPD; Gillings and Holley, 1997a, 1997b), ERIC-PCR has been used to monitor the structural dynamics of a single community and to compare the microbial structure of related communities (Di Giovanni *et al.*, 1999; Wei *et al.*, 2004; Yan *et al.*, 2007). In the HFA piglet gut, ERIC-PCR fingerprinting showed that a complex microbiota had established by 5 days of age and became stable at 12 days (Supplementary Figure 1). At 12 days of age, all of the HFA littermates

exhibited similar DNA fingerprints of gut microbiota, implying a similar microbial population composition in all piglets (Figure 1a). The establishment of a genetically uniform microbiota in all the HFA piglets is a critical feature of this animal model. Reproducibility of human flora colonization in piglets was verified by analyzing three litters of animals (n=28) inoculated with a fecal suspension from the same donor. The gut community DNA fingerprints of piglets in all three litters were highly similar to each other (Figure 1b).

Figure 1:
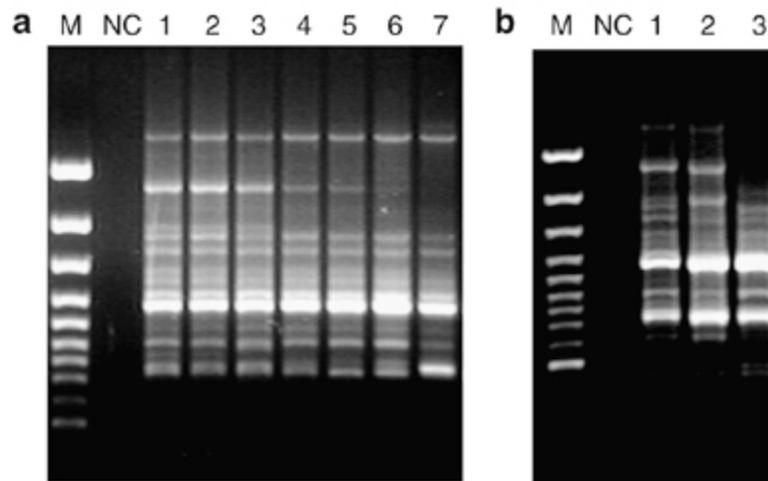
ERIC-PCR fingerprints of fecal flora in HFA piglets. (**a**) Seven HFA littermates at 12 days of age. (**b**) Three HFA piglets, one from each of three litters. Lane M: DNA size marker (100-bp ladder). Lane NC: negative control. ERIC-PCR, enterobacterial repetitive intergenic consensus sequence-PCR; HFA, human flora-associated.

Although a complex microbiota was established in piglet gut with minimal individual variation, it was essential to determine whether this microbiota simulated that of the human donor. To compare the bacterial composition in the gut of HFA piglets, the human donor and conventionally raised (CV) piglets, ERIC-PCR fingerprinting-based DNA hybridization was employed to compare the population structure in different communities based on DNA sequence composition. Figure 2a shows the ERIC-PCR fingerprints of intestinal microbiota in 10 unrelated healthy human individuals, five CV piglets and two HFA piglets. The patterns indicated that each individual had a complex and host-dependent microbiota. Clustering analysis (Figure 2b) based on the fingerprints showed that all human and HFA piglet samples clustered together and the CV piglet samples clustered in another group, which indicated that the DNA fingerprints of HFA piglets were more similar to that of humans than to the CV piglets.

Direct analysis of the ERIC-PCR fingerprints will inevitably overestimate the similarity of different communities since the DNA separation is based only on the size of the DNA fragments rather than on the actual sequence (Wei *et al.*, 2004). To reveal the sequence differences in the fingerprints of the 17 samples tested, community DNA hybridization was performed using all of the ERIC-PCR amplicons of the human donor (individual H_4 in Figure 2a), as probes for hybridization with all the samples (Figure 2c). Strong hybridization was detected in most of the human samples (except individual H_3), whereas the five CV piglet samples all produced weak signals. The two HFA piglets showed strong hybridization with 6–8 fragments shared with the donor fingerprint. The hybridization signals of the HFA piglets were even stronger than those of most other human individuals. This result indicated that though the DNA fingerprints of the

established flora in the HFA piglets was not completely identical to that of the human donor, the DNA sequence composition which represented the microbial composition of HFA piglets most closely resembled that of the human donor. The same strategy of ERIC-PCR fingerprinting-based community DNA hybridization has been successfully used to compare the microbial composition of human gut microbiota to discover genomic sequences as physical markers associated with healthy gut (Wei *et al.*, 2004) and to identify functional relevant microbial populations in a coking wastewater treatment system (Yan *et al.*, 2007).

Figure 2:
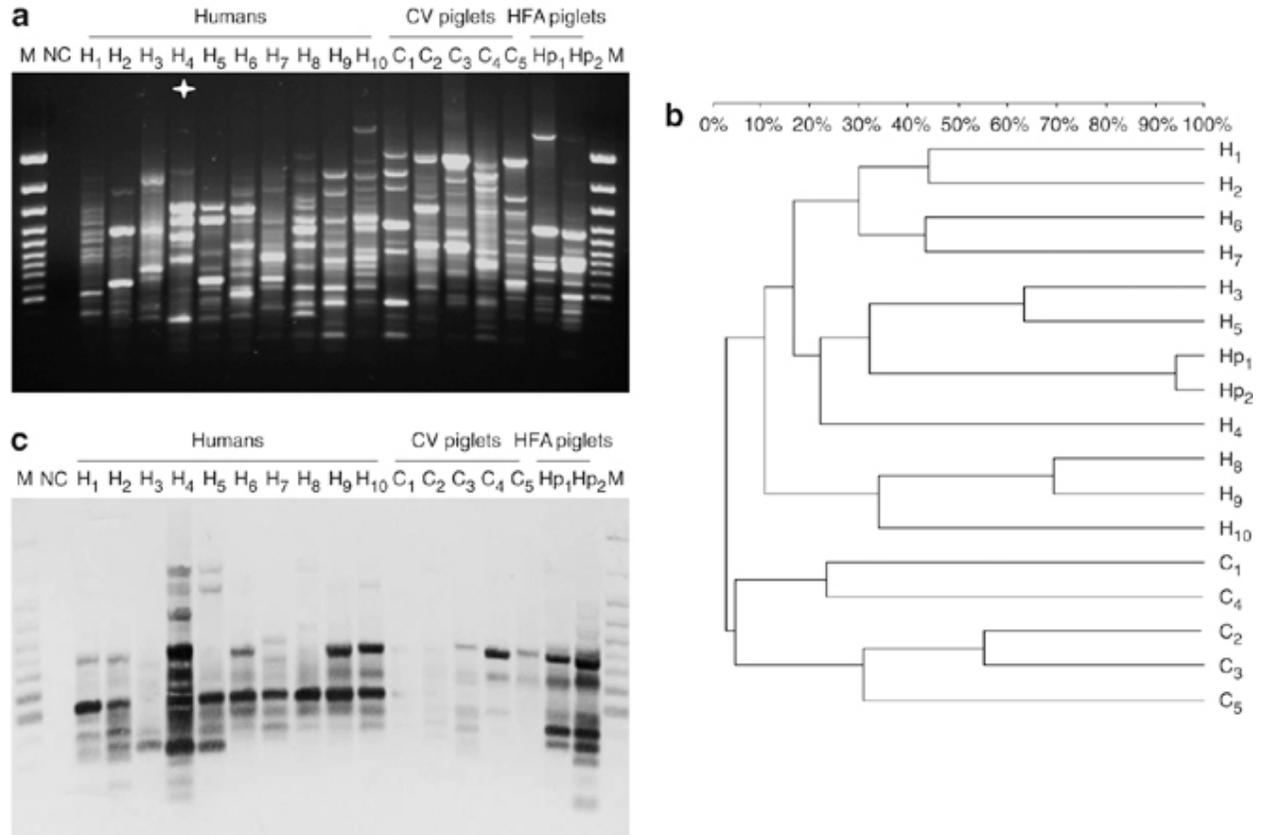
Comparison of microbiota in different humans, conventional piglets and HFA piglets via ERIC-PCR-based community DNA hybridization. (**a**) ERIC-PCR fingerprints of fecal flora of 10 different human beings (H_1–H_{10}), five conventional piglets (C_1–C_5) and two HFA piglets (Hp_1 and Hp_2). (**b**) Dendrogram illustrating the similarity correlation of fingerprints in (**a**). (**c**) Southern blot using DIG-labeled ERIC-PCR amplicons of individual H_4 as mixed probe to hybridize with the PCR products shown in (**a**). Individual H_4 was the human donor for the HFA piglets. Lane M: DNA size marker (100-bp ladder). Lane NC: negative control. ERIC-PCR, enterobacterial repetitive intergenic consensus sequence-PCR; HFA, human flora-associated.

In the highly diverse microbial communities, the universal bacterial primer-based PCR-DGGE/TGGE fingerprinting is of low resolution and results in a profile too complex to investigate the bacterial population structure in detail or to monitor the shifts in microflora composition. Many group-specific PCR-based DGGE/TGGE approaches have been developed to target the predominant or relatively less abundant but potentially important species in complex microbial communities (Heuer *et al.*, 1997; Kowalchuk *et al.*, 1998; Walter *et al.*, 2000; Satokari *et al.*, 2001; de Silva *et al.*, 2003; Garbeva *et al.*, 2003; Pang *et al.*, 2005). These methods increased the sensitivity of DGGE/TGGE detection to allow visualization of interspecies relationships and abundance of the bacterial group in a highly diversed background.

In this study, two important human gut bacterial groups in HFA piglets, bifidobacteria and *Bacteroides* spp, were analyzed by group-specific TGGE methods (Figure 3). Bifidobacteria is the third most common genus in the human intestine, constituting about 3% of the adult fecal flora and more than 75% of the infant fecal flora (Langendijk *et al.*, 1995; Franks *et al.*, 1998; Harmsen *et al.*, 2000). Moreover, some strains of bifidobacteria have been used as probiotics to modulate the human intestinal ecosystem due to the many health-promoting properties of these bacteria (Picard *et al.*, 2005). *Bacteroides* spp is the most dominant group of the normal indigenous flora in the human gut comprising more than 25% of the total bacteria in human feces (Wilson *et al.*, 1997; Suau *et al.*, 1999; Sghir *et al.*, 2000).

Figure 3:
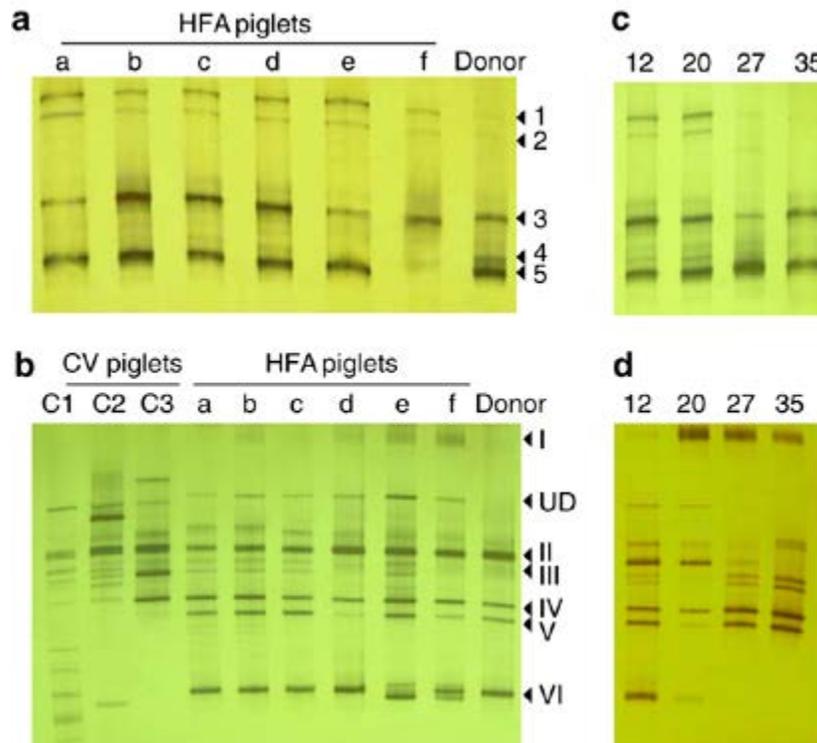
Comparison of TGGE patterns for bifidobacteria (**a**) and *Bacteroides* (**b**) in six HFA piglets (a–f, two randomly selected piglets per litter), the donor, and three conventional piglets (C1–C3). (**c** and **d**) showed the succession of bifidobacteria (**c**) and *Bacteroides* (**d**) in HFA piglets from 12 to 35 days of age. TGGE, temperature gradient gel electrophoresis; HFA, human flora-associated.

Bifidobacteria TGGE profiles of the human donor exhibited five discernable bands (bands 1–5), and the species identity of each band was determined by sequencing and co-migration analysis (see Supplementary Figure 2). The DNA bands corresponded to the following sequences: band 1, *Bifidobacterium bifidum* (100% homologous); band 2, *B. infantis* (100% homologous); band 4, *B. adolescentis* (98% homologous); band 5, *B. pseudocatenulatum* (100% homologous). Band 3 contained two sequences, one was a new species whose sequence was only 95% homologous with *B. bifidum* and the other was 99% homologous with *B. longum*. For all CV piglet samples, no bifidobacteria-specific PCR amplicon was detected. According to the literature, the population of bifidobacteria in pigs was low (<1% total bacteria) or even undetectable (Brown *et al.*, 1997; Leser *et al.*, 2002; Mikkelsen *et al.*, 2003). And the species isolated from porcine samples such as *B. suis*, *B. globosum*, *B. pseudolongum*, *B. thermophilum*, *B. boum* and *B.*

choerinum were also different from the species generally found in the human gut (Mikkelsen *et al.*, 2003; Simpson *et al.*, 2003). On the contrary, bifidobacteria-specific amplicons were obtained from all HFA piglet samples and the TGGE patterns of HFA piglets were very similar to that of the human donor, indicating that most of the donor's bifidobacteria species had colonized the intestinal tract of HFA piglets (bands 1–3 and 5; Figure 3a). The total bifidobacteria count in the gut of HFA piglets was 8.41 ± 0.21 $\log_{10}$ per gram of feces (wet weight) as enumerated by real-time PCR, compared with 8.10 ± 0.04 $\log_{10}$ in the donor. Levels of bifidobacteria in the HFA piglets were similar to those in the donor.

Bacteroides species were detected in all samples of CV piglets, HFA piglets and the human donor. The species identity of each band in TGGE gel of the donor has been described previously (Pang *et al.*, 2005). The microbiota of the donor was comprised of 31.6% *B. vulgatus*-like species, 13.2% *B. caccae*-like species, 13.2% *B. uniformis*, 5.3% *Bacteroides* sp AR20, 2.6% *B. fragilis* and 34.2% potentially new *Bacteroides* species. HFA piglets yielded TGGE patterns similar to each other as well as to the human donor, but remarkably different from CV piglets (Figure 3b). However, profiles of the HFA piglets were not completely identical to those of the donor. Two products (Figure 3b band UD and III) produced very weak signals in the donor fingerprints, but were much stronger in the HFA piglet fingerprints. In addition, several new bands appeared in the HFA piglet patterns. Such shift of the relative abundance of the lineages of transplanted microbiota in the new host has been reported in the study of reciprocal microbiota transplantation between zebrafish and mouse (Rawls *et al.*, 2006). Real-time PCR of *Bacteroides* showed consistent population sizes in HFA piglets and the donor (9.31 ± 0.31 $\log_{10}$ and 9.33 ± 0.02 $\log_{10}$ per gram of feces, respectively).

We also observed that introduction of solid food during the weaning period significantly alters gut microbiota in HFA piglets. Species of bifidobacteria and *Bacteroides* showed clear succession during weaning (19–25 days of age) in HFA piglets (Figure 3c and d) with a significant decline in *B. bifidum* and *B. infantis* (bands 1 and 2). *B. bifidum* and *B. infantis* are predominant in the microbiota of infants, especially breast-fed infants, although they are detected at low levels in adults (Sakata *et al.*, 2005). Thus for bifidobacteria, HFA piglets showed a human-like ecological succession.

This work demonstrated that transplantation of gut flora from human to piglets is feasible. The resultant flora more closely resembles that of the human donor than of other humans or CV pigs. Two important bacterial groups, bifidobacteria and *Bacteroides*, successfully colonized the guts of the recipient piglets. This HFA piglet model provides a new opportunity for research in gut ecology, nutrition and metabolism. And it also has a unique ability to assess, manipulate and investigate the contributions of gut microbiota to individualized drug responses. Therefore, such HFA piglets can be used as a tool to screen new drugs, investigate toxicity of relevant pharmacological compounds, and study the mechanisms of their activities.

REFERENCES

Backhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A *et al*. (2004). The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci USA* 101: 15718–15723.

Bartosch S, Fite A, Macfarlane GT, McMurdo MET. (2004). Characterization of bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients by using real-time PCR and effects of antibiotic treatment on the fecal microbiota. *Appl Environ Microbiol* 70: 3575–3581.

Brown I, Warhurst M, Arcot J, Playne M, Illman R, Topping D. (1997). Fecal numbers of bifidobacteria are higher in pigs fed *Bifidobacterium longum* with a high amylose cornstarch than with a low amylose cornstarch. *J Nutr* 127: 1822–1827.

Clayton TA, Lindon JC, Cloarec O, Antti H, Charuel C, Hanton G *et al*. (2006). Pharmaco-metabonomic phenotyping and personalized drug treatment. *Nature* 440: 1073–1077.

de Silva KRA, Salles JF, Seldin L, van Elsas JD. (2003). Application of a novel *Paenibacillus*-specific PCR-DGGE method and sequence analysis to assess the diversity of *Paenibacillus* spp in the maize rhizosphere. *J Microbiol Methods* 54: 213–231.

Di Giovanni GD, Watrud LS, Seidler RJ, Widmer F. (1999). Fingerprinting of mixed bacterial strains and BIOLOG gram-negative (GN) substrate communities by enterobacterial repetitive intergenic consensus sequence-PCR (ERIC-PCR). *Curr Microbiol* 38: 217–223.

Franks AH, Harmsen HJM, Raangs GC, Jansen GJ, Schut F, Welling GW. (1998). Variations of bacterial populations in human feces measured by fluorescent *in situ* hybridization with group-specific 16S rRNA-targeted oligonucleotide probes. *Appl Environ Microbiol* 64: 3336–3345.

Garbeva P, van Veen JA, van Elsas JD. (2003). Predominant *Bacillus* spp in agricultural soil under different management regimes detected via PCR-DGGE. *Microb Ecol* 45: 302–316.

Garthoff LH, Henderson GR, Sager AO, Sobotka TJ, O'Dell R, Thorpe CW *et al*. (2002). The autosow raised miniature swine as a model for assessing the effects of dietary soy trypsin inhibitor. *Food Chem Toxicol* 40: 487–500.

Gerard P, Beguet F, Lepercq P, Rigottier-Gois L, Rochet V, Andrieux C *et al*. (2004). Gnotobiotic rats harboring human intestinal microbiota as a model for studying cholesterol-to-coprostanol conversion. *FEMS Microbiol Ecol* 47: 337–343.

Gillings M, Holley M. (1997a). Amplification of anonymous DNA fragments using pairs of long primers generates reproducible DNA fingerprints that are sensitive to genetic variation. *Electrophoresis* 18: 1512–1518.

Gillings M, Holley M. (1997b). Repetitive element PCR fingerprinting (rep-PCR) using enterobacterial repetitive intergenic consensus (ERIC) primers is not necessarily directed at ERIC elements. *Lett Appl Microbiol* 25: 17–21.

Harmsen HJ, Wildeboer-Veloo AC, Raangs GC, Wagendorp AA, Klijn N, Bindels JG *et al*. (2000). Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. *J Pediatr Gastroenterol Nutr* 30: 61–67.

Hazenberg MP, Bakker M, Verschoor-Burggraaf A. (1981). Effects of the human intestinal flora on germ-free mice. *J Appl Bacteriol* 50: 95–106.

Heuer H, Krsek M, Baker P, Smalla K, Wellington EM. (1997). Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. *Appl Environ Microbiol* 63: 3233–3241.

Hirayama K. (1999). Ex-germfree mice harboring intestinal microbiota derived from other animal species as an experimental model for ecology and metabolism of intestinal bacteria. *Exp Anim* 48: 219–227.

Hooper LV, Gordon JI. (2001). Commensal host–bacterial relationships in the gut. *Science* 292: 1115–1118.

Hulton CSJ, Higgins CF, Sharp PM. (1991). ERIC: a novel family of repetitive elements in the genome of *Escherichia coli*, *Salmonella typhimurium* and other enterobacteria. *Mol Microbiol* 5: 825–834.

Imaoka A, Setoyama H, Takagi A, Matsumoto S, Umesaki Y. (2004). Improvement of human faecal flora-associated mouse model for evaluation of the functional foods. *J Appl Microbiol* 96: 656–663.

Kelly D, Campbell JI, King TP, Grant G, Jansson EA, Coutts AGP *et al*. (2004). Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. *Nat Immunol* 5: 104–112.

Kowalchuk GA, Bodelier PLE, Heilig GHJ, Stephen JR, Laanbroek HJ. (1998). Community analysis of ammonia-oxidising bacteria, in relation to oxygen availability in soils and root-oxygenated sediments, using PCR, DGGE and oligonucleotide probe hybridisation. *FEMS Microbiol Ecol* 27: 339–350.

Langendijk P, Schut F, Jansen GJ, Raangs GC, Kamphuis GR, Wilkinson MH *et al*. (1995). Quantitative fluorescence *in situ* hybridization of *Bifidobacterium* spp. with genus-specific 16S rRNA-targeted probes and its application in fecal samples. *Appl Environ Microbiol* 61: 3069–3075.

Leser TD, Amenuvor JZ, Jensen TK, Lindecrona RH, Boye M, Moller K. (2002). Culture-independent analysis of gut bacteria: the pig gastrointestinal tract microbiota revisited. *Appl Environ Microbiol* 68: 673–690.

Ley R, Turnbaugh PJ, Klein S, Gordon JI. (2006). Microbial ecology: human gut microbes associated with obesity. *Nature* 444: 1022–1023.

Mallett AK, Bearne CA, Rowland IR, Farthing MJ, Cole CB, Fuller R. (1987). The use of rats associated with a human faecal flora as a model for studying the effects of diet on the human gut microflora. *J Appl Bacteriol* 63: 39–45.

Mikkelsen LL, Bendixen C, Jakobsen M, Jensen BB. (2003). Enumeration of bifidobacteria in gastrointestinal samples from piglets. *Appl Environ Microbiol* 69: 654–658

Nicholson J, Holmes E, Wilson ID. (2005). Gut microorganisms, mammalian metabolism and personalized health care. *Nat Rev Microbiol* 3: 431–438.

Oozeer R, Goupil-Feuillerat N, Alpert CA, van de Guchte M, Anba J, Mengaud J *et al*. (2002). *Lactobacillus casei* is able to survive and initiate protein synthesis during its transit in the digestive tract of human flora-associated mice. *Appl Environ Microbiol* 68: 3570–3574.

Pang X, Ding D, Wei G, Zhang M, Wang L, Zhao L. (2005). Molecular profiling of *Bacteroides* spp. in human feces by PCR-temperature gradient gel electrophoresis. *J Microbiol Methods* 61: 413–417.

Parracho HM, Bingham MO, Gibson GR, McCartney AL. (2005). Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children. *J Med Microbiol* 54: 987–991.

Picard C, Fioramonti J, Francois A, Robinson T, Neant F, Matuchansky C. (2005). Review article: bifidobacteria as probiotic agents-physiological effects and clinical benefits. *Aliment Pharmacol Ther* 22: 495–512.

Raibaud P, Ducluzeau R, Dubos F, Hudault S, Bewa H, Muller MC. (1980). Implantatin of bacteria from the digestive tract of man and various animals into gnotobiotic mice. *Am J Clin Nutr* 33: 2440–2447.

Rawls J, Mahowald MA, Ley RE, Gordon JI. (2006). Reciprocal gut microbiota transplants from zebrafish and mice to germ-free recipients reveal host habitat selection. *Cell* 127: 423–433.

Roland N, Rabot S, Nugon-Baudon L. (1996). Modulation of the biological effects of glucosinolates by inulin and oat fibre in gnotobiotic rats inoculated with a human whole faecal flora. *Food Chem Toxicol* 34: 671–677.

Sakata S, Tonooka T, Ishizeki S, Takada M, Sakamoto M, Fukuyama M *et al*. (2005). Culture-independent analysis of fecal microbiota in infants, with special reference to *Bifidobacterium* species. *FEMS Microbiol Lett* 243: 417–423.

Satokari RM, Vaughan EE, Akkermans AD, Saarela M, de Vos WM. (2001). Bifidobacterial diversity in human feces detected by genus-specific PCR and denaturing gradient gel electrophoresis. *Appl Environ Microbiol* 67: 504–513.

Sghir A, Gramet G, Suau A, Rochet V, Pochart P, Dore J. (2000). Quantification of bacterial groups within human fecal flora by oligonucleotide probe hybridization. *Appl Environ Microbiol* 66: 2263–2266.

Simpson PJ, Stanton C, Fitzgerald GF, Ross RP. (2003). Genomic diversity and relatedness of bifidobacteria isolated from a porcine cecum. *J Bacteriol* 185: 2571–2581.

Suau A, Bonnet R, Sutren M, Godon JJ, Gibson GR, Collins MD *et al*. (1999). Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut. *Appl Environ Microbiol* 65: 4799–4807.

Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 444: 1027–1031.

Versalovic J, Koeuth T, Lupski JR. (1991). Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Res* 19: 6823–6831.

Walter J, Tannock GW, Tilsala-Timisjarvi A, Rodtong S, Loach DM, Munro K *et al*. (2000). Detection and identification of gastrointestinal *Lactobacillus* species by using denaturing gradient gel electrophoresis and species-specific PCR primers. *Appl Environ Microbiol* 66: 297–303.

Wang RF, Cao WW, Cerniglia CE. (1996). PCR detection and quantitation of predominant anaerobic bacteria in human and animal fecal samples. *Appl Environ Microbiol* 62: 1242–1247.

Wei G, Pan L, Du H, Chen J, Zhao L. (2004). ERIC-PCR fingerprinting-based community DNA hybridization to pinpoint genome-specific fragments as molecular markers to identify and track populations common to healthy human guts. *J Microbiol Methods* 59: 91–108.

Wilcks A, van Hoek AHAM, Joosten RG, Jacobsen BBL, Aarts HGM. (2004). Persistence of DNA studied in different *ex vivo* and *in vivo* rat models simulating the human gut situation. *Food Chem Toxicol* 42: 493–502.

Wilson KH, Ikeda JS, Blitchington RB. (1997). Phylogenetic placement of community members of human colonic biota. *Clin Infec Dis* 25: S114–S116.

Yan X, Xu Z, Feng X, Liu Y, Liu B, Zhu C *et al.* (2007). Cloning of environmental genomic fragments as physical markers for monitoring microbioal populations in coking wastewater treatment system. *Microb Ecol* 53: 163–172.