Characterization of normal microbial communities in weanling pigs using 16S rRNA gene sequences

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Culture-dependent methods of bacterial identification have proven inadequate for characterizing the gastrointestinal microbial ecosystem because these methods are time-consuming and reflect only culturable species. To overcome these limitations, 16S ribosomal RNA analysis has become a preferred approach for cataloging members of diverse communities. 16S rRNA-encoding genes occur in all prokaryotes, and, while highly conserved, usually contain sufficient variation to assign sequences to specific species. Prior studies have generated a large database of sequence information, aiding in the phylogenetic characterization of the intestinal bacterial population in humans and swine. The objective of this study was to characterize the normal microbial community in the GI tract of weanling pigs. The subjects of this study, three pigs fed a commercial diet and raised indoors, were sacrificed at 5 weeks of age. Bacteria isolated from the harvested chyme were processed to extract genomic DNA, which was then used as a template for PCR. The ~1.5 kb PCR product was then ligated into a pGEM T Vector and used to transform *E. Coli* JM109 cells. White colonies were harvested from plated cultures and grown in overnight liquid cultures. Plasmid DNA was extracted from the overnight cultures, and the presence of inserts was confirmed by digestion with EcoRI. The DNA sequences were determined and subjected to a BLAST search to determine the respective phylogenetic positions. Intestinal microbial dynamic was approximated by observing sequence prevalence of individual species.