Anaerobe 45 (2017) 31–39

Contents lists available at ScienceDirect

Anaerobe

journal homepage: www.elsevier.com/locate/anaerobe

Early exposure to agricultural soil accelerates the maturation of the early-life pig gut microbiota

Nguyen Vo [a, b], Tsung Cheng Tsai [c], Charles Maxwell [c], Franck Carbonero [a, b, *]

[a] Department of Food Science, University of Arkansas, 2650 North Young Avenue, Fayetteville, AR, 72704, USA
[b] Interdisciplinary Graduate Program in Cell and Molecular Biology, University of Arkansas, Fayetteville, AR, USA
[c] Department of Animal Science, University of Arkansas, 1120 West Maple, Fayetteville, AR, 72701, USA

ARTICLE INFO

Article history:
Received 2 November 2016
Received in revised form
23 February 2017
Accepted 24 February 2017
Available online 27 February 2017

Handling Editor: Laura M Cox

Keywords:
Gut microbiota
Hygiene hypothesis
Early-life
Agricultural soil

ABSTRACT

Reduced microbial exposure in early childhood is postulated to be associated with subsequent immune deficiencies and associated health conditions. This corollary to the "hygiene hypothesis" has grown of popularity in the medical field, but can only be really tested with animal models. Based on previous observation that access to outdoor environment improves piglets' growth performance, we simulated early microbial exposure by providing pigs with topsoil during the lactation phase. Specifically, pigs from 20 litters were assigned to either control treatments (C) or soil treatments (S): pigs exposed to topsoil from day 4 postpartum to the end of lactation. At weaning, five unisex littermates of 10 sows from each treatment were penned together and grew up in the same conditions. Fecal samples were collected at on d 13 (Lactation: L), 21 (Weaning: WT), 35 (Mid-nursery, MNT), 56 (End of Nursery: EONT) and 96 (End of Growth: EGT) for 16s rRNA amplicon high-throughput sequencing. Overall, common trends of gut microbiota maturation, associated with diet switch from maternal milk to plant-based diet, were observed. *Bacteroides*, *Clostridium* XIVa and Enterobacteriaceae were most abundant during lactation, while *Prevotella*, *Megasphaera* and *Blautia* became abundant after weaning. Remarkably, exposure to soil resulted in a faster maturation of the piglets gut microbiota at weaning, while a completely distinct phase was observed at day 35 for control piglets. Soil-exposed piglets tened to harbor a more diverse gut microbiota at weaning and day35, however the more significant changes were at those time points in terms of composition. *Prevotella*, and a wide range of Firmicutes members were significantly enriched in soil-exposed piglets from the lactation to the end of nursery phase. It can be hypothesized that those taxa were either directly transmitted from the soil or stimulated by the presence of plant material in the soil. Those changes were accompanied by depletion in several potentially harmful taxa, as well as improved growth performance between weaning and the end of nursery phase. Our findings suggest that early exposure to soil strongly influences the maturation of the early-life piglets, probably allows for a better adaptation to the plant-based diet, and possibly improves overall health.

1. Introduction

As the importance of the gut microbiota in health and disease was rediscovered [20,25,34], the hygiene hypothesis [49] has become increasingly popular [1,7,8]. Importantly, the human microbiota, especially the gut microbiota, has been increasingly reported as the probable main parameter, leading to suggestions to change the hypothesis name to "microbial exposure" or "microbial deprivation" [61]. Since one of the corollaries of the hypothesis is the observation that modern hygiene and westernization appear to drive the recent rise in so-called western diseases, there have been several recent studies focused on determining the "primitive" human microbiome. There is now substantive evidence that populations that are still living ancestral way of life harbor higher gut microbial diversity with the presence of distinguishing genera/species [19,36–38,40,43,47]. However, it should be noted that the majority of studies only include one time-point sampling, and rarely children [21,26,28,57], thus the dynamics of the "natural" early-life human gut microbiota remain elusive.

* Corresponding author. Department of Food Science, University of Arkansas, 2650 North Young Avenue, Fayetteville, AR, 72704, USA.
E-mail addresses: nguyenvo@uark.edu (N. Vo), ttsai@uark.edu (T.C. Tsai), cmaxwell@uark.edu (C. Maxwell), fgcarbon@uark.edu (F. Carbonero).

http://dx.doi.org/10.1016/j.anaerobe.2017.02.022
1075-9964/

Early-life human gut microbiome has been studied quite extensively in relation to mode of delivery [14,45] and nutrition (breastfeeding vs formula) [41,53] and to a lesser extent to antibiotic and pre-probiotic exposure [17,52]. These variables and sometimes the gut microbiome have been associated with disease risk [1–5,24]. Since it is clearly not possible to set up studies with controlled environmental (hygiene and cleanliness) variables with infants or children, any data available relies on self-reported parameters, often broad in scope. For instance, there have been reports that exposure to environments with arguably higher microbial diversity has a negative relationship with atopic sensitizations and asthma prevalence [13,44]. It is commonly postulated that disruption of "normal" microbiota leads to allergic and other auto-immune diseases through impaired immunological development [10,12,16,18,31,48].

As it is commonly the case in medical-oriented studies, the vast majority of studies attempting to link gut microbiota and immune status rely on rodent models (mice in the overwhelming majority). While this choice of model is supported by its advantages for short-term well controlled studies, it is well known that any findings cannot be directly translated to conclusions relevant to human biology and health. An emerging animal model is the swine that is characterized by closer similarity to human in terms of size, digestive physiology and metabolic processes [22] and specifically intestinal microbiota composition [59]. Indeed, there are several reports of promising simulation of early-life human gut microbiota, as well as gut microbiota/immune system interaction using swine models. In addition of being a medical model, gut microbiota dynamics in early-life piglets has recently been reported to potentially affect subsequent health and more generally growth performance, a crucial outcome in the context of animal production.

In the present study, we simulated the outdoor rearing-like environment by exposing piglets to topsoil during lactation (since day 4 to day 13 postpartum). Our objective was to investigate the potential impact of early exposure to environmental soil microbes on the gut microbiota composition through time and between treatments and overall growth performance.

2. Method

2.1. Experimental design – exposure of top soil to pigs

Piglets (PIC-29 x 380) from 20 litters (litter size>10) were assigned to be either managed conventionally in farrowing crates (C) or daily exposed to topsoil (S) from 4 days postpartum (d 0) to the end of lactation (d 17). Approximately 1 kg of topsoil from Tontitown, AR (Sod Store, INC.) was placed and maintained throughout the 17 day into flat containers in S pigs farrowing crates for unrestricted rooting.

At weaning, unisex littermates were penned together in groups of 10 (total of 10 pens and 50 pigs per treatment), and were all subjected to identical growing conditions (no more soil exposure) until marketing. Pigs were fed common antibiotic-free corn-SBM-DDGS nutrient-adequate diets, and individual pigs body weight (BW), was measured at birth and weaning (d 17), together with feed disappearance at each phases change (d 25, 38, 56, 77, 100, 123 and 142), and again at the end of trial (d 163) to determine the growth performance. Fresh grab fecal samples for gut microbiota sequencing were collected on d 13 (Lactation: L), 21 (Weaning: WT), 35 (Mid-nursery, MNT), 56 (End of Nursery: EONT) and 96 (End of Growth: EGT).

Ten samples from each pre-selected animals were taken for each treatment at mentioned time and were processed for microbiota analyses except for L phase. In L, we collected 8 samples for C treatments and 12 samples for S treatments.

2.2. 16s rRNA sequencing for gut microbiota composition

Fecal samples received from the Animal Science Department (University of Arkansas, AR, USA) were stored at −70 Celsius for subsequent processing steps.

We extracted DNA using the QIAamp® Fast DNA Stool Mini kit (Qiagen) following the manufacturer's instructions with addition of a bead-beating step, as commonly advised [60]. Specifically, we weighted around 180 mg-220 mg fecal samples into autoclaved tubes containing 100 mg of each 0.1 mm and 0.5 mm diameter Zirconia-silicate beads (BioSpec Products) and the InhibitEX Buffer provided in the kit. The tubes were then subjected to bead-beating at 5.5 m/s for 60 s in a Fastprep®-24 bead beater. To obtain more concentrated DNA, we added 50 μl of Buffer ATE to dilute in the last step instead of 200 μl as stated in the manufacturer's instruction. DNA quality and quantity were checked by gel electrophoresis and Nanodrop and/or Qbit (ThermoFisher) measurement.

For library preparation, we followed the dual-index amplicon sequencing approach developed by Ref. [27] with slight modifications. Briefly, DNA extracts were used as template for PCR using the dual index primers targeting the V4 region of the 16S rRNA gene sequence in 96 well plates according to the scheme. PCR conditions were as follows: initial denaturation (2 min at 95 °C) 25 amplification cycles (95 °C for 30s, 55 °C for 30s, 72 °C for 1 min) and final elongation (72 °C for 5 min). A random row was submitted to gel electrophoresis to confirm successful amplification, and all amplicons were subjected to purification and normalization using SequalPrep™ Normalization Plate Kit (ThermoFisher). Amplicons were pooled and the pool subjected to library quality control: quantitative PCR by NEBNext® Library Quant Kit for Illumina® (New England Biolabs) and TapeStation Bioanalyzer (Aligent) following manufacturer recommendations. Because resulting libraries were found to have unexpected additional bands, gel extraction of the correct band was performed with the QIAquick Gel Extraction Kit (Qiagen) and resulting libraries submitted again to quality control. Based on previous sequencing runs on our MiSeq, libraries were diluted to 6pM and pooled with PhiX (internal sequencing standard; 5%) and sequenced on an Illumina MiSeq using the Illumina MiSeq® Reagent Kit v2 (500 cycle). The runs were monitored with Sequence Analysis Viewer with particular emphasis on appropriate cluster density (700-800 k/mm^2) and quality scores (final > Q30 score of >70%). When satisfactory sequencing was obtained, preliminary sequence analysis was performed on BaseSpace (illumina) with the 16S Metagenomics application. Resulting Fastq files were readily demultiplexed and assigned to samples and downloaded for bioinformatics analyses.

Sequences were processed following the MiSeq SOP (https://www.mothur.org/wiki/MiSeq_SOP). Briefly, sequences were screened and aligned to the Silva database for 16S RNA gene sequences. Subsequently, OTUs were picked and assigned to taxonomic groups. Resulting OTUs and taxonomic tables were exported to Excel sheets for basic analyses and JMP and PAST software for univariate (T-test, ANOVA) and multivariate (ANOSIM, PcoA, NMDS) statistical analyses. We used subsampled data to calculate observed OTUs, Chao, Shannon and Inverse Simpson index via Mothur [46] and compared results by non-parametric Wilcoxon test using JMP software.

3. Results

From 102 samples, a total of 5194218 raw sequences were obtained of which 2995037 high quality reads were used for the analyses. Samples yielding less than 2807 high-quality reads were discarded; the remaining samples analyzed had an average of 29950 reads. All reads were analyzed together in the Mothur