

## EFFECT OF GENOTYPE AND AGE ON A DEFINED MICROBIOTA IN GNOTOBIOTIC SCID PIGLETS

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### Abstract

Severe combined immunodeficient (SCID) individuals lack functional T and B lymphocytes, leading to a deficient adaptive immune system. SCID pigs are a unique large animal biomedical model as they possess many similarities to humans, allowing for the collection of translatable data in regenerative medicine, cancer, and other biomedical research topics. While many studies suggest early gut microbiota development is necessary for developing the intestinal barrier and immune system, these animals are often cesarian section derived, leaving them uncolonized for normal intestinal microflora. The hypothesis was that an increase in complexity of microbiota inoculum will allow for more stability in the composition of the gut microbiota of SCID piglets. This was tested across multiple litters of SCID piglets with three different defined microbiota consortium (2-strain, 6-strain, 7-strain). All piglets received their designated defined microbiota by oral gavage immediately after birth and again 24 hours later. There was no effect of SCID genotype on the composition of the gut microbiota, but there was a significant effect due to piglet age. Additionally, all three defined microbiota consortia were deemed safe to use in SCID piglets, and the 7-strain microbiota was the most stable over time. Based on these results, the 7-strain defined microbiota will be added to the SCID pig husbandry protocol, allowing for a more reproducible model.

### 1 Introduction

Severe combined immunodeficient (SCID) animal models have become important tools in biomedical research. The SCID genotype can be produced naturally due to mutations or by genetic modification of specific genes. These mutations lead to a SCID phenotype where T and B cells are deficient or non-functional (Cossu, 2010). Due to their compromised immune system, SCID animals allow for studies in cancer therapies, drug trials, and xenotransplants, making them a valuable component of pre-clinical research (Boettcher et al., 2018; Singer et al., 2019).

SCID mice are well established models that are cheaper and easier to care for than a large animal SCID model; however, research involving mice does not always translate efficiently to human medicine (Uhl & Warner, 2015). Boettcher and colleagues (2019) demonstrated that tumors grown in SCID mice were phenotypically different from the human tumor the cells were

derived, while the tumors grown in the SCID pigs were much more similar. The pig genome is another area that is more similar to humans than the mouse genome (Junhee Seok et al., 2013; Wernersson et al., 2005). These similarities lead to the expectation that using a SCID pig model would allow for better data translation to human medicine. Additionally, in specific disciplines, such as tissue generation research on repair of cardiac disease, the FDA states that data obtained from small animal models is insufficient (FDA, 2009). This regulation demonstrates the benefit of a SCID pig model in biomedical research as opposed to SCID mice.

SCID pigs can occur naturally due to mutation in the Artemis gene, which is essential for DNA repair during the stages of development for T and B lymphocytes. When two mutations occur on different alleles of the Artemis gene ( $ART^{-/-}$ ) in piglets, the resulting phenotype is a deficiency in T and B cells with functioning natural killer (NK) cells (Waide et al., 2015). Genetic modification is another method to create SCID pigs. While there are many different methods to create genetically modified SCID pigs, this article highlights the use of a mutation in the IL-2 receptor subunit gamma chain ( $IL2RG^{-Y}$ ), which is necessary for lymphoid development. The resulting phenotype is a deficiency in T and NK cells with the presence of B cells that are not functional (Suzuki et al., 2012).

SCID pigs require a large, specific pathogen free (SPF) environment for long term survival (Lee et al., 2014), an example of which was previously described (Powell et al., 2018). Snatch farrowing was utilized to obtain the SCID piglets in aseptic manner prior to entry into the housing environment. Snatch farrowing involves catching piglets as they exit the vaginal canal preventing piglets from encountering contaminated surfaces (Huang et al., 2013). This method had some challenges, as the vaginal microbiota can be host to opportunistic pathogens, risking infection in the piglets. There was a change in protocol to avoid this risk of infection and it involves the use of cesarian section (Boettcher et al., 2020). The cesarian section method does not allow SCID piglets to receive microbiota from the sow like they would with a vaginal delivery (Wang et al., 2013), leading to an uncolonized gut where opportunistic pathogenic bacteria can thrive. The lack of colonization presents challenges, as it is known that early colonization of the gut is essential for piglet health (Kelly et al., 2007; Olszak et al., 2012). We tested three different defined microbiota that varied in complexity over multiple litters of SCID piglets and hypothesized that the most complex defined microbiota would be the most stable in the gut microbiome of the piglets.

With the knowledge of the significance of a colonized gut for piglet health, alternatives to snatch farrowing were sought, including providing the cesarian-derived SCID piglets with an artificial microbiome. We created three defined microbiota consortia, each with increased complexity. The selected bacteria in the defined microbiota were adapted from a defined commensal microbiota (DMF) designed to mimic the infant gut (Huang et al., 2018). This study aimed to determine the effect of genotype and piglet age on the defined microbiota in the gut of SCID piglets. Additionally, the goal was to create a safe, defined microbiota that could be implemented into our husbandry protocol to aid in the reproducibility of the SCID pig model.

## 2 Materials and Methods

### 2.1 Ethics statements

All procedures involving animals were conducted after approval by the Iowa State University Institutional Animal Care and Use Committee.

## 2.2 Experimental Animals

Yorkshire-Landrace gilts were raised on site at the Iowa State University campus. These females were artificially inseminated with thawed, frozen semen previously collected from boars also raised at Iowa State University. Specific genotypes used for each litter are discussed below.

Sows were moved at day 114 of gestation to the Iowa State University Laboratory Animal Resource (LAR) facility. They were prepared for cesarean section by Iowa State University LAR care staff, which included washing the sow.

Sows for cesarean section were sedated with intramuscular acepromazine (MWI Animal Health, Boise, ID) in the housing room 30-45 minutes prior to moving to the surgery preparation room. Once transported to the preparation room, sows received 0.2 mg/kg intramuscular morphine sulphate (Spectrum Chemical, New Brunswick, NJ) and topical lidocaine cream (Alembic Pharmaceuticals Ltd., Vadodara, Gujarat, India) was applied to the ears and epidural site. After approximately 10-15 minutes contact time for the lidocaine cream, about 35 ml of lidocaine (MWI Animal Health, Boise, ID) was administered via epidural, as described (Swindle & Smith, 2015). An ear catheter was placed (Kaiser-Vry et al., 2023) and propofol (Zoetis Inc., Kalamazoo, MI) administered IV to effect (500-800 mg). Once a sufficient anesthetic plane was achieved, the sow was intubated, and anesthesia maintained on isoflurane administered with oxygen. The surgical site was shaved, and an initial scrub performed in the preparation room.

The sow was moved to the surgery room and placed in lateral recumbency on the surgery table. The sow was maintained on IV fluids of Lactated Ringers Solution +/- 5% dextrose (Baxter International, Deerfield, IL) for the duration of the surgery. Once properly positioned, a complete surgical scrub was performed. Spray-on adhesive was used to secure a homemade flexible film surgical isolator to the sow. The surgical isolator and contents had been previously autoclaved where possible (surgical instruments and metal transfer cylinder) and sterilized with Clidox spray (Pharmaceutical Research Laboratories, Waterbury, CT). The surgical isolator allowed gloved access for the surgeon and surgery assistant as well as four people to revive piglets. A paramedian incision was made into the abdominal cavity and the uterus exteriorized. Incisions were made over each piglet, as each piglet was removed the umbilicus was clamped, and 1-2 drops naloxone (Somerset Therapeutics LLC, Hollywood, FL) placed on the tongue to reverse the anesthetic allowing piglets to be revived more promptly. Piglets were stimulated to assist with revival, warmed with towels and handwarmers. Any piglet that was still sedated approximately 10 minutes after removal from the uterus, received an additional 1-2 drops naloxone. The sow was euthanized with Fatal-Plus Solution (Vortech Pharmaceuticals, Dearborn, MI) after all piglets had been removed. Once all piglets were stable, piglets were moved to a transfer cylinder for transport to their housing environment.

The isolators used for designated litters were previously described (Aluthge et al., 2020). The BioBubble biocontainment housing environment for SCID pigs and the sterilization protocol of the biocontainment environment have also been previously detailed (Powell et al., 2018). A modification to the previously described sterilization protocol was the addition of C. diff Solution Tablets (3M, Athlone, Ireland). The tablets were dissolved in water and, using a foam sprayer, was sprayed on all surfaces in the bubble. After all surfaces air dried, the room was sprayed down with water. This change to the sterilization protocol was added prior to the third animal trial discussed in this article.

Once piglets were received into their selected environment, they were processed. All piglets received one milliliter of Gleptoforte injectable pig iron (Ceva Animal Health, Services, Lenexa, KS) intramuscularly, had needle teeth clipped, temperature programmable microchips

(Unified Information Devices, Lake Villa, IL) injected sub-dermally for identification purposes, temperatures collected via microchips, and umbilical cords tied off with suture. A small piece of umbilical tissue was collected using scissors, which were disinfected with Oxivir Tb Wipes (Diversity, Fort Mill, SC) between collections, then placed in sterile 1.5 ml microcentrifuge tubes. Genotyping was performed using the DNeasy Blood and Tissue Kit (Qiagen, Germantown, MD) to purify genomic DNA and determine the genotype of the piglets. As genotyping results take more than a day to be returned, a preliminary method to rapidly determine which piglets were SCID and non-SCID was utilized. One milliliter of blood was drawn from the jugular vein of each piglet and a complete blood count (CBC) was performed to look at lymphocyte levels of each piglet. Within the experimental herd at ISU, we have found that if a piglet has less than 1.0 lymphocytes per microliter it is very likely to be SCID, which allows placing an animal in a treatment group when immediately necessary. An initial fecal swab was also collected prior to feeding and microbiota inoculation. The rest of the fecal swab collection protocol will be discussed in the sample collection section.

After piglets were processed, the microbiota consortium samples were given by oral gavage catheterization. Five milliliters of inoculum were syringed through the catheter followed by five milliliters of colostrum to ensure all inoculum was flushed through the catheter into the stomach. This process was then performed again approximately 24 hours later.

After the initial fecal swab was collected, irradiated colostrum powder (Bovine IgG Calf's Choice Total Gold Colostrum, SCCL, Saskatoon, Saskatchewan) was mixed with sterile water and piglets were fed using baby bottles. The goal was to feed 20 to 25 milliliters of colostrum to each piglet, every thirty minutes to provide up to 250-300 milliliters in the first 36 hours. The amount of colostrum the piglets received at each feeding was recorded. The SCID piglets received 100% colostrum by care staff for the first 36 hours of life. A bowl of colostrum was added into the piglet's environment at 12 hours of life to encourage drinking on their own. Once a piglet was observed drinking from the bowl, they were no longer offered the bottle. After 36 hours, piglets started receiving a mixture containing 50% colostrum and 50% milk replacer (irradiated Birthright Pig Milk Replacer powder (Ralco, Marshall, MN) appropriately mixed with water) until day 7. From one week to three weeks of age, piglets received a mixture of 25% colostrum and 75% milk replacer. Table 1 outlines the feeding timeline protocol.

Throughout the first 72 hours, piglets were continuously monitored. Piglet temperatures were recorded every hour to monitor for signs of infection and ensure the environment was an appropriate temperature for the piglets. If any abnormal behavior was noted, an LAR on-call veterinarian was alerted and further action was directed (Powell et al., 2018). Piglets were weighed once a day to ensure they were gaining weight appropriately.

The litters included for analysis were chosen based on husbandry similarities and their genetic heritage. The only major change between litters is the defined microbiota inoculum they received (Table 2).

### **2.2.1 Litter 17**

A double heterozygous dam ( $ART^{+/-} IL2RG^{+/-}$ ) was bred to an Artemis heterozygous sire ( $ART^{+/-}$ ). This breeding resulted in two  $ART^{-/-}$  SCID piglets (A-SCID), two  $IL2RG^{-/-}$  SCID piglets (G-SCID), and one non-SCID pig used for this project. Piglets were placed into isolators for the duration of this trial. All piglets received the 2-strain microbiota inoculum.

### **2.2.2 Litter 23**

A heterozygous dam for the Artemis gene ( $ART^{+/-}$ ) was bred to the same sire described in litter 17 ( $ART^{+/-}$ ). Offspring from this breeding were two A-SCIDs and two non-SCID pigs

used for this project. Like litter 17, this litter resided in isolators for the duration of this trial. All four piglets received the 6-strain microbiota inoculum.

### 2.2.3 Litters 33 and 34

The dams used to create litters 33 and 34 were double heterozygous for the Artemis gene ( $ART^{+/-} IL2RG^{+/-}$ ). These females were bred to a double mutant (BMT)-rescued SCID male ( $ART^{-/-} IL2RG^{-/Y}$ ). This SCID male was born in a litter created by embryo transfer then had a bone marrow transplant performed which led to the reconstitution of T, B, and NK cells (Boettcher et al., 2020). Piglets for this trial were placed into the BioBubble biocontainment room and remained there for the duration of the trial.

The total number of piglets born across these two litters was 20 piglets. A fecal swab was collected during day one processing from all 20 piglets, to determine gnotobiotic status of the piglets. For the overall analysis, we only used piglets that met all timepoints we wanted to include. This included two A-SCIDs, five G-SCIDs, and one non-SCID pig. All piglets received the 7-strain microbiota consortium that had an increased concentration of bacteria, compared to the other two groups. The 7-strain microbiota inoculum contained  $\sim 10^8$  CFU/mL for each isolate while the 2-strain and 6-strain inoculum contained  $\sim 10^6$  CFU/mL for each isolate.

Environmental swabs were collected and analyzed for this trial to determine if environmental bacterial contaminants were present. These swabs were collected prior to the entry of piglets and again from the same location approximately six hours later. Samples were collected by swabbing a 10 cm x 10 cm section of all four floor locations in the bubble, the cart in which supplies were stored and the buses where the piglets are raised.

### 2.3 Development of the 2, 6, and 7-strain Inocula

*Clostridioides difficile* is a frequent member of neonatal pig gut microbiota and is present in two-thirds of litters and one-third of piglets (Songer, 2004). Using the snatch farrowing method mentioned above, the piglet's microbiota was largely acquired from the sow and the farrowing environment (Boettcher & Tuggle, 2022). This method resulted in numerous cases of *C. difficile* infection which prompted a switch to sterile cesarean delivery and the development of a defined microbiota that protected against *C. difficile* proliferation. Huang and colleagues (2018) designed a defined commensal microbiota (DMF) for gnotobiotic piglets, to represent the infant gut microbiota, to further their studies (Huang et al., 2018). Their methods guided us towards adapting their DMF to a defined microbiota for use in SCID pig.

### 2.4 Cultivation of the inoculum

All handling and incubation steps were completed in anaerobic conditions (5/5/90%  $H_2/CO_2/N_2$  Mixed Gas, Airgas, Radnor, PA).  $-80^\circ C$  stock cultures (preserved with either 20% glycerol or 7% DMSO) of the isolates were plated on the media listed in Table 3. De Man – Rogosa – Sharpe (MRS) agar (Thermo Fisher Scientific, 52.0 g/L, + 20 g/L agar), Brain Heart Infusion (BHI) agar (Thermo Fisher Scientific, 37 g/L, + 20 g/L agar), and BHI + 1% Yeast Extract (BD, 10 g/L + 20 g/L agar) agar plates were pre-reduced in anaerobic conditions prior to use. Anaerobic liquid media was prepared following the methods described (Uchino & Ken-ichiro, 2013).

Culture purity was routinely confirmed by sequencing the 16S rRNA gene of each isolate. To obtain genomic DNA, a small scraping of cells from an agar plate was suspended in 20  $\mu L$  of 10 mM EDTA in a microtube. After vortexing, the microtube was heated at  $100^\circ C$  for 10 minutes. The microtube was then centrifuged for 10 minutes at  $14.6 \times g$ . After centrifugation, 15  $\mu L$  of supernatant was transferred to a new microtube containing 75  $\mu L$  of PCR-grade diethyl pyrocarbonate (DEPC) water. 16S rRNA gene PCR was completed using the primers 616F (5' –

AGA GTT TGA TYM TGG CTC – 3') and 1492R (5' – GGY TAC CTT GTT ACG ACT T – 3') following the standard Platinum II Taq Hot-Start DNA polymerase protocol (Thermo Fisher Scientific). The PCR products were confirmed using agarose gel electrophoresis, then purified using the Thermo Scientific GeneJET PCR Purification Kit (Thermo Fisher Scientific). The purified DNA was sent to the ISU DNA facility where it underwent Sanger sequencing. The resulting 16S rRNA gene sequences were assigned to known species using NCBI BLAST. The top match for each isolate can be found in Table 3.

The 2-strain and 6-strain inocula were created using the following method. For each isolate individually, a 10  $\mu$ L loopful of cells was used to inoculate 10 mL of pre-reduced broth (corresponding media type for each isolate listed in Table 3). The liquid cultures were incubated for 20 hours at 37°C yielding  $\sim 10^8 - 10^9$  CFU/mL. To check for contamination, 10  $\mu$ L of each liquid culture was used to inoculate agar plates in triplicate. The plates were incubated in aerobic and anaerobic conditions for 48 hours at 37°C and monitored for growth. If abnormal colony morphologies were observed, the identities of the potential contaminants would be confirmed by sequencing the 16S rRNA gene. Cultures were discarded if contamination was found. The clean liquid cultures were then combined in a sterile 500 mL bottle and diluted with an equivalent volume of BHI broth. Sterile glycerol was then added to reach a 20% (v/v) glycerol solution. Aliquots of the mixed inoculum were stored in 50 mL centrifuge tubes at -80°C. Prior to use, the tubes were thawed at 37°C for 10 minutes and were administered by oral gavage within 30 minutes of thawing.

Once thawed, the surviving count of each isolate was  $\sim 10^6$  CFU/mL. For the 7-strain inoculum, the initial bacterial load was increased to  $\sim 10^8$  CFU/mL for each isolate. To achieve this, 2.5 mL of the initial 10 mL broth cultures were used to inoculate 50 mL of broth (Table 3). The 50 mL cultures were incubated at 37°C for 36 hours yielding  $\sim 10^8 - 10^9$  CFU/mL. To confirm culture purity, samples were plated on solid media as described above. The liquid cultures were then mixed in a sterile disposable 1 L plastic bottle, and sterile glycerol was added to reach a 20% (v/v) glycerol solution. 25 mL aliquots of the mixed inoculum were stored in 50 mL centrifuge tubes at -80°C.

## 2.5 Sample collection

Swabs were collected at six timepoints: while piglets were being processed after cesarean section, prior to microbiota inoculation which is designated as day 0, 24 hours after the second inoculation of microbiota, three days after the second inoculation, seven days after the second inoculation, and 14 days after the second inoculation. See Table 4 for the sample collection timeline.

Puritan Fecal Opti-Swabs Elongated Tip Swab, 2 ml Cary Blair Medium (Harmony, Garden Grove, CA) were used for sample collection. Fecal swabs were inserted into the anus, taking care to not touch anywhere else, until the tip was fully inserted, approximately 2 cm. The swab was then gently spun, removed from the anus, inserted into the collection vial, and tightly secured. Immediately after collection, the fecal swabs were stored at -80 °C until DNA extractions were performed. Fecal swabs collected during piglet processing were streaked on BHI media to determine gnotobiotic status.

Samples collected up to day 15 were used for analysis. These were the last swabs used for analysis as weaning occurs after this collection time point and dry feed is introduced to the piglets, which is known to change the gut microbiota composition (Frese et al., 2015; Guevarra et al., 2019). Fecal swabs were not used in the analysis if they were taken after piglets received antibiotics.

## 2.6 Processing of samples and DNA extraction

Fecal swabs were removed from the freezer, thawed at room temperature, and vortexed for 10 minutes to detach fecal material from the swab. Lids were then removed from the vials, and the swab was removed from the vial using forceps. The swab was discarded, and the cap replaced. Between each swab, forceps were dipped in bleach and rinsed in sterile deionized water to avoid cross contamination. Fecal swab tubes were then centrifuged for three minutes at 4,694 x g at 4°C (Cassas et al., 2024). The supernatant was then discarded, being careful not to disturb the pellet.

DNA extraction from the fecal swabs was then performed following the protocol provided in the DNeasy Powerlyzer Powersoil Kit (Qiagen). Upon completion of the DNA extraction protocol, the concentration of the samples was determined using a ND-100 Nanodrop spectrophotometer (NanoDrop Technologies, Dockland, DE). Many of the samples, especially from the earlier timepoints, were found to have low DNA concentrations (~ 10 ng/μl or less). To ensure the DNA could be amplified prior to amplicon sequencing, samples with the lowest DNA concentrations underwent 16S rRNA gene PCR. Successful amplification was confirmed with agarose gel electrophoresis. Although many samples had low DNA concentrations, all samples showed DNA amplification allowing them to be submitted for Illumina MiSeq 16S rRNA gene amplicon sequencing. Samples with DNA concentrations higher than 25 ng/μL were diluted to reach the desired concentration of 25 ± 5 ng/μL.

The processing method described above was the same for all three trials and litters. Additional control samples were added to the DNA extraction process for Litter 33 and Litter 34 samples. An unused fecal swab that was never frozen and an unused fecal swab that was frozen at -80°C were included for DNA extraction at the end of each kit as controls and were processed like the rest of the samples. This was to determine if contaminants were present in the blank fecal swabs. Two samples of PCR-grade DEPC water were also added to the end of the plate submitted for sequencing, to determine if there were any contaminants contracted during sequencing.

## 2.7 16S rRNA gene amplicon sequencing and data processing

16S rRNA gene amplicon sequencing of the DNA obtained from SCID piglet fecal swabs was completed using the Illumina MiSeq platform at the ISU DNA facility. The ISU DNA facility performed custom library preparation to amplify the 16S rRNA gene V4 region. Universal 16S rRNA gene bacterial primers 515F (5'-GTGYCAGCMGCCGCGGTAA-3') and 806RB (5'-GGACTACNVGGGTWTCTAAT-3'), , and the Platinum Hot Start PCR Master Mix (2x) (Thermo Fisher Scientific) were used to amplify the V4 variable region with the following thermocycler conditions: initial denaturation step at 94 °C for 3 min; 45 s of denaturing at 94 °C; 60 s of annealing at 50 °C; 90 s of extension at 72 °C. This was repeated for 35 cycles and finished with a 10 min extension at 72 °C. After PCR, equal amounts of amplicons from each sample were pooled into a single tube and purified using the standard protocol of the UltraClean PCR Clean-Up Kit (MO BIO Laboratories). All described reagents were dispensed using a Mantis robot (Formulamatrix). The barcoded amplicons then underwent paired-end (2 x 250) 500-cycle sequencing on an Illumina MiSeq platform.

The 16S rRNA gene amplicon sequences for litters 17 and 23 were processed and analyzed separately from litters 33 and 34 using the following protocol. Raw sequences were processed using mothur (v1.43.0) (Schloss et al., 2009) following a protocol based on the MiSeq Standard Operating Procedure (Kozich et al., 2013). The “make.contigs” command was used to merge and filter the paired-end reads. Parameters including a maximum homopolymer run of

eight bp, a minimum length of 252 bp, and a cutoff of zero ambiguities were applied for the “screen.seqs” command. Sequences were aligned using the SILVA reference database (v138) (Quast et al., 2013) and the “align.seqs” command. Chimeric sequences were removed using the “chimera.vsearch” command in combination with the SILVA.gold reference. *De novo* operational taxonomic unit (OTU) clustering at 99% gene similarity was completed and the resulting OTUs were classified using the SILVA reference database (v138). The resulting OTU data was imported into R where the package decontam (v1.20.0) (Davis et al., 2018) was used to identify and remove contaminating DNA. Microbial community visualization was completed using the Phyloseq (v1.38.0) (McMurdie & Holmes, 2013), vegan (v2.5.7) (Oksanen et al., 2022), and ggplot2 (v3.4.0) (Wickham, 2016) R packages.

## 2.8 Statistical Analysis

OTUs with fewer than 10 reads were removed prior to statistical analysis. The microbial raw abundance data was normalized to minimize sequencing depth biases by dividing by the corresponding sample library size, resulting in “relative abundance” values used for further analysis. The following model was used to evaluate the effects of genotype and time on the beta diversity and alpha diversity of the piglet’s microbiota:

$$Y_{ijk} = \mu + g_i + \tau_j + g_i\tau_j + \varepsilon_{ijk}$$

Where  $Y_{ijk}$  is the observed value for  $k^{\text{th}}$  experimental unit within the  $i^{\text{th}}$  level of genotype (A-SCID, G-SCID, or non-SCID) at the  $j^{\text{th}}$  timepoint (Day 2, Day 4, Day 7-8, or Day 14-15);  $\mu$  is the overall mean;  $g_i$  is the fixed effect of the  $i^{\text{th}}$  genotype ( $i = \text{A-SCID, G-SCID, or non-SCID}$ );  $\tau_j$  is the fixed effect of the  $j^{\text{th}}$  timepoint ( $j = \text{Day 2, Day 4, Day 7-8, or Day 14-15}$ );  $g_i\tau_j$  is the interaction of genotype and timepoint; and  $\varepsilon_{ijk}$  is the error as described by the model for  $Y_{ijk}$ .

Differences in beta diversity were analyzed using Bray-Curtis distances and visualized using principal coordinate analysis (PCoA) plots using the Phyloseq package in R. A permutational multivariate analysis of variance (PERMANOVA) and a permutational multivariate analysis of dispersion was completed with the commands “adonis2” and “betadisper” from the vegan package in R (Oksanen et al., 2022). The resulting p-values were adjusted to account for multiple-comparisons using Bonferroni’s correction (Dunn, 1961).

The Phyloseq package in R was used to generate alpha diversity measurements for the number of observed species, Chao species richness, Simpson evenness, and Shannon diversity. In addition to the described model, Pig ID was included as a repeated measure to account for the covariance among the samples taken from the same animal. The least square means (LSmeans) of all alpha diversity measurements were compared using the PROC MIXED procedure in SAS (Version 9.4, SAS Inst., Cary, NC). The resulting p-values from the pairwise comparisons were corrected for multiple-comparisons using Tukey’s Honest Significant Difference test (Tukey, 1949) Adjusted p-values were considered significant if  $p < 0.05$ .

## 3 Results

### 3.1 Description of strains used in the defined microbiota and sample collection

To determine which defined microbiota was most stable in the gut microbiome, SCID piglets were c-sectioned and inoculated with different versions of a defined microbiota. The complexity of the microbiota was gradually increased over time, starting with a 2-strain microbiota, then a 6-strain microbiota, and finally a 7-strain microbiota. The list of strains included in each inoculum can be found in Table 3. We sought to balance the risk associated with opportunistic infections from otherwise normal porcine microflora while simultaneously mitigating the negative effects of an absent gastrointestinal microbial community.



*Peptacetobacter spp.*, applied in the 2-strain and 7-strain inoculum, is a novel bacterial strain that converts primary bile acids. To avoid potential overgrowth of a *Peptacetobacter spp.* monoculture, *Lactococcus lactis* was included in the 2-strain inoculum. Research has shown that *L. lactis* is beneficial for piglets post-weaning, resulting in improved growth performance and intestinal immunity among other benefits (Yu et al., 2021). In the 6 and 7- strain inocula, *Lactobacillus brevis*, *Bifidobacterium adolescentis*, *Bifidobacterium longum*, and *Clostridium clostridioforme*, were efficacious as part of a defined commensal microbiota, or DMF as described by Huang and colleagues (2018), in a neonatal gnotobiotic pig model. *Bacteroides thetaiotaomicron* was also included in the described DMF, but *Bacteroides vulgatus* was substituted based on availability from the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL).

As outlined above, fecal swabs were collected from SCID piglets in each litter as part of processing after birth and these samples were denoted as day 0. Fecal samples were then collected according to the protocol outlined in Table 4. Samples collected up to day 15 were included in statistical analysis as weaning occurs after this timepoint. Litter 17, however, only included samples collected up to day 8 as antibiotics were introduced prior to collection at the last time point. Environmental swabs were also collected from the BioBubble biocontainment facility to confirm sterility of the housing environment prior to piglet entry. DNA extraction and 16S rRNA amplicon sequencing were then performed for all collected swabs.

### **3.2 Combined analysis of sample, control, and environment swabs led to exclusion of day 0 samples from further analysis**

Figure 1 is a combined Principal Coordinate Analysis (PCoA) plot which includes data from all fecal swab collections for litters 17, 23, 33, and 34, controls samples, environmental samples and swabs collected from the sows of Litter 33 and 34. For statistical purposes, samples collected from Litter 17 and 23 were grouped and labeled with the same color and Litter 33 and 34 samples were grouped and labeled with the same color. This means control swabs from Litter 17 and 23 were labeled together as one item and the same for day 0 swabs and piglet's swabs. The same labeling system was followed for Litter 33 and 34 samples. Day 0 swabs were the swabs initially collected from the piglets and the data points labeled as "piglets" were the swabs collected after this initial timepoint. Environment swabs were labeled as "env" in Figure 1 and the location of collection for these swabs is provided in the materials and methods. The samples labeled as controls are DEPC water samples to test for contaminants during DNA extraction. The samples labeled as sow, were collected from the anus and vulva from the sows of Litter 33 and 34. Lastly, the samples labeled as water control were the DEPC water samples included at the end of the plate submitted for 16S rRNA gene amplicon sequencing.

At day 0, prior to microbiota inoculation, piglets are expected to be free of microorganisms; however, results from 16S rRNA amplicon sequencing revealed a minimal number of reads, with an average of 30,892 reads pre-decontam package (discussed in materials and methods), from these swabs. These reads could indicate the piglets were not germ free at the start of the experiment. Since the data indicated a low presence of bacterial DNA in these samples, we analyzed day 0 swabs separately from the later timepoints. The purpose was to look at the relationship between day 0 swabs, the non-day 0 swabs labeled as "piglets", the environmental swabs, and the control swabs. We found that the day 0 swabs, environmental swabs, and control swabs clustered together, and separately from the non-day 0 piglet swabs. This indicated the read counts present for day 0 samples was likely background, indistinguishable from low levels of bacterial DNA present in the environment or process control

swabs, and that no bacteria were present in the piglets or the environment. Thus, day 0 swabs were then omitted from future microbiome analysis.

There was a significant difference between the non-day 0 swabs and the day 0 and control swabs ( $P=0.001$ ) that were detected using PERMANOVA. There was also a significant difference between Litter 17 and 23 swabs, labeled a “piglets\_17.23”, compared to Litter 33 and 34, labeled as “piglets\_33.34”, ( $P=0.001$ ) detected using PERMANOVA. With these results, we removed the day 0 samples from analysis and the Litter 17 and 23 were analyzed separately from Litter 33 and 34.

Supplementary Figure 1 is an additional PCoA plot displaying only data collected during the third trial (litter 33 and 34). With the addition of environmental swabs and more control swabs, we wanted to identify the beta diversity of samples from this trial. This figure displays a clear difference in clustering of day 0, control, and environmental swabs when compared to the non-day 0 swabs denoted as “piglets” in the figure. Furthermore, the environmental and day 0 swabs collected from litter 33 and 34 were cultured, shortly after collection, to determine if any bacteria were present. This included six environmental swabs collected prior to piglet entry and all twenty swabs collected from the piglets on day 0. Two blank fecal swabs were also included to confirm their sterility. Each sample had one plate for aerobic growth and one plate for anaerobic growth. The environmental swabs collected prior to piglet entry, cultured in an aerobic environment, resulted in three plates with no growth, two plates with a single colony, and one plate that grew many colonies with many morphologies. The results from culturing in the anaerobic environment were two plates with no growth, one plate with three colonies, two plates that had multiple colonies, and one plate that had many colonies with many morphologies. Of the day 0 piglet swabs, one piglet sample out of twenty presented a single colony in aerobic conditions, it is possible this was contamination from collection the sample. The plates for the blank swabs did not have any growth. The cultivation results and addition of controls reinforced the decision to exclude day 0 swabs from further analysis.

### **3.3 No significant effect of SCID piglet genotype on beta diversity of piglet gut microbiota, regardless of piglet age**

Figure 2 contains unconstrained PCoA plots for litter 17 (Figure 2A), litter 23 (Figure 2B), and litter 33 and 34 (Figure 2C) to examine the beta diversity of the microbiota of SCID piglets due to genotype, regardless of piglet age, due to the defined microbiota they received. Whole bacterial community beta diversity comparisons were performed using PERMANOVA for all groups.

Litter 17 (2-strain microbiota inoculum) PERMANOVA results ( $P = 0.018$ ) revealed a significant difference due to genotype. There were no significant pairwise comparisons noted in an attempt to determine specific genotype effects, which could be due to lack of power from the small sample size. The second trial, involving litter 23 (6-strain microbiota inoculum), revealed significant differences due to genotype using PERMANOVA ( $P = 0.028$ ). A significant PERMDISP2 ( $P=0.009$ ) test showed that there was unequal variance between the treatment groups, meaning assumptions for PERMANOVA testing were not met. Lastly litter 33 and 34 (7-strain microbiota inoculum) PERMANOVA results ( $P=0.340$ ) revealed no significant differences due to genotype.

### **3.4 Significant effect of piglet age on beta diversity of gut microbiota of SCID piglets, regardless of genotype.**

Figure 3 examines beta diversity of the gut microbiota of SCID piglets, regardless of genotype. PERMANOVA results for litter 17 ( $P = 0.001$ ), litter 23 ( $P=0.008$ ), and litter 33 and 34 ( $P= 0.001$ ) revealed a significant difference due to piglet age over time.

Pairwise comparisons for Litter 17 (Figure 3A) revealed a significant difference between day 4 and day 7-8 ( $P=0.027$ ). Litter 23 (Figure 3B) did not have any significant pairwise comparisons noted for a direct comparison of piglet age, presumably due to lack of power from a small sample size. Lastly, litter 33 and 34 (Figure 3C) pairwise comparisons revealed a significant difference between day 2 and day 14-15 ( $P=0.018$ ) and a significant difference between day 4 and day 14-15 ( $P=0.012$ ).

### **3.5 Increase in inoculum complexity and concentration appears to offer greater microbiota stability over time, regardless of genotype**

Based on 16S rRNA gene amplicon sequencing, the relative abundance of each inoculated bacteria for all timepoints from the three trials can be seen in Figure 4. This aids in the understanding of how the microbiome composition changes, over the sampling timepoints.

Litter 17 received the 2-strain inoculum which included *Peptacetobacter spp.* and *Lactococcus lactis*. At day 2, both bacteria were present, with the relative abundance of *L. lactis* being the greatest. A shift is seen at day 4 where the relative abundance of *Peptacetobacter spp.* and other bacteria increased. At day 7-8, the relative abundance of *L. lactis* had decreased significantly, *Peptacetobacter spp.* decreased slightly, and other bacteria increased. By day 7-8, other bacteria, that were not part of the 2-strain inoculum, were most abundant.

Litter 23 received the 6-strain inoculum which included *Bacteroides vulgatus*, *Bifidobacterium adolescentis* and *Bifidobacterium longum* (which were grouped together in Figure 4), *Clostridium clostridioforme* (denoted as *Lachnoclostridium* in Figure 4, as this is the SILVA Taxonomy name), *Lactobacillus brevis*, and *Lactococcus lactis*. At day 2, the relative abundance of *L. lactis* was greatest followed by *B. vulgatus*. *Bifidobacterium* and *L. brevis* were present in small percentages and other bacteria that was not inoculated were also present. At day 4, the relative abundance of *L. lactis* and *B. vulgatus* increased while there was a decrease in *Bifidobacterium*, *L. brevis* and other bacteria. At day 7-8, *B. vulgatus*, *Bifidobacterium* and other bacteria increased in relative abundance, and *L. lactis* decreased. By day 14-15, the relative abundance of *B. vulgatus*, *L. clostridioforme* and other bacteria had increased, while there was a major decrease in *L. lactis*. *Bifidobacterium* abundance was unchanged. Other, not inoculated, bacteria appear to flourish by day 14-15; however, compared to the 2-strain microbiota at day 7-8, the relative abundance of inoculated species increased when using the 6-strain inoculum.

Lastly, Litter 33 and 34 received the 7-strain inoculum which included all bacteria in the 6-strain as well as *Peptacetobacter spp.*, which was seen in the 2-strain. As well, a 100x increase in the concentration of the bacteria in this inoculum compared to the prior two was used. At day 2, which is after the second microbiota inoculation, the majority of the relative abundance was other bacteria; however, *B. vulgatus*, *L. clostridioforme* and *L. lactis* were present. By day 4, the relative abundance of *B. vulgatus* and *L. clostridioforme* increased while *L. lactis* and other bacteria decreased. Day 7-8 composition was like day 4 with the addition of *Peptacetobacter spp.*. Finally, by day 14-15, the relative abundance of *Peptacetobacter spp.*, *L. clostridioforme*, and *Bifidobacterium* increased while *L. lactis* and *B. vulgatus* had decreased. The percentage of other bacteria varied little. *L. brevis*, was inoculated as part of the 7-strain defined microbiota but was not present in any of the relative abundance timepoints in Figure 4.

The relative abundance of other, not inoculated, bacteria was greater at day 2 when inoculated with the 7-strain, compared to day 2 for the 2-strain and 6-strain inoculum. However, the other three timepoints for the 7-strain inoculum were most consistent to each other compared to the other two groups.

#### 4 Discussion

There has been minimal literature published using SCID pig models due to the recent discovery and creation of such models, as well as complexities involved in the rearing of these highly disease-susceptible pigs. It is known that early establishment of the gut microbiota is important for the development of a healthy gut microbiota, which also is known to have a significant interaction with the immune system (Kelly et al., 2007). To our knowledge, a defined microbiota has not been used in a protocol for rearing SCID piglets, but defined microbiota consortia have been used in other animal model systems, such as gnotobiotic pigs (Huang et al., 2018; Laycock et al., 2012) We investigated three different defined microbial consortia and the effect of SCID genotype and piglet age have on the gut microbial composition of piglets pre-weaning. The bacterial strains used for these consortia were adapted from a DMF previously used to create an environment that mimicked the infant gut microbiota (Huang et al., 2018).

The primary purpose of this study was to test different levels of complexity of defined microbiota consortia and determine if there was an effect on the gut microbiota due to genotype or piglet age. A secondary outcome from the results of this study was to develop a defined microbiota that was safe for use in SCID piglets and that could be standardized and reproduced easily.

All litters were delivered by cesarean section, then transported to their sterile housing environment where they received their respective defined microbiota consortia (2-strain, 6-strain, and 7-strain) by oral gavage. Fecal swabs were collected according to the protocol outlined in the materials and methods. Day 0 swabs were not included in the analysis due to their similarity to control and environmental swabs, indicating there was little to no live bacteria present in the day 0 samples (Figure 1).

The composition of the litter 33 gut microbiota compared to the litter 34 gut microbiota was not found to be significantly different. Because of this, and the fact that the litters were born on the same day, had a common environment, and received the same 7-strain inoculum, litter 33 and 34 data were combined for analysis. Along with being the most complex inoculum, the 7-strain defined microbiota inoculum also had the highest concentration of bacteria at  $\sim 10^8$  CFU/mL while the 2-strain and 6-strain defined microbiota inoculum had a concentration of  $\sim 10^6$  CFU/mL. The consortia developed by Huang and colleagues (2018), utilized in gnotobiotic piglets, had a concentration of about  $7 \times 10^5$  CFU/mL. With their positive outcome, we utilized a higher concentration at  $2 \times 10^6$  CFU/mL for the 2-strain defined microbiota and  $6 \times 10^6$  CFU/mL for the 6-strain (one million of each bacteria per mL of inoculum). Cheng and colleagues (2022) designed a complex defined microbiota for use in mice at a concentration of  $10^9$  CFU/mL for each bacterial strain, with a total of  $10^{10}$  bacteria in the 0.2 mL inoculum. This prompted the increase in total bacterial concentration for the 7-strain defined microbiota at  $7 \times 10^8$  CFU/mL; with a total of  $3.5 \times 10^9$  CFU in the 5 mL inoculum. Thus, this increase was thought to bring the inoculum closer to the equivalent dose given to mice (Cheng et al., 2022). However, since these mice were immunocompetent, an important goal with the increase in bacterial concentration of the 7-strain inoculum was to determine if it was safe for use in SCID piglets. Additionally, this increase was thought to be closer to what the equivalent dose would be in comparison to what is

given to mice, given the large difference in intestinal size (Cheng et al., 2022). All three defined microbiota consortia (2-strain, 6-strain, 7-strain) were determined safe for use in the SCID piglets, including the 7-strain with the increase concentration of bacteria, as the inoculated bacteria was never found in blood collected for culture.

#### **4.1 Piglet genotype does not have a significant effect on piglet gut microbiota**

For all three inoculum, a significant effect due to genotype on the microbial composition was not significant. Litter 17, which received the 2-strain microbiota inoculum, had a significant p-value for whole community level statistics for the effect of genotype across all genotypes on the beta diversity of the piglet gut microbiota. However, there were no significant ( $P > 0.05$ ) pairwise comparisons between specific genotypes. This is likely due to a lack of statistical power. Due to antibiotic administration prior to collection of the day 15 swab, because of confirmed infection in this litter, this time point was not included in analysis based on the knowledge that antibiotics affect the gut microbial composition (Gao et al., 2018). This decreased the already small sample size.

For Litter 23, our evaluation of the effect of genotype on the bacterial community composition was inconclusive. The two groups (A-SCID and non-SCID) had unequal variance, and thus, not all necessary assumptions were met for the PERMANOVA test. Genotype was not found to have a significant effect on the beta diversity of the litters that received the 7-strain inoculum (Litters 33 and 34).

Statistical analysis of alpha diversity further confirmed the lack of effect of genotype on the piglet microbiota, as assessed by beta diversity analysis (Supplementary Figure 2). The young age of the piglets used in the study could contribute to this indifference. We did observe a significant difference in alpha diversity of piglet microbiota due to piglet age for all three treatment groups (Supplementary Figure 3). Prior research has shown that there is a decrease in alpha diversity of the gut microbiota for SCID mice compared to non-SCID mice (Zheng et al., 2019). It is important to note that these SCID mice had a different mutation leading to their SCID phenotype than the pigs used in our study. These mice were nonobese diabetic (NOD)/SCID mice which have low, but detectable, natural killer cell activity. This decrease in alpha diversity was found over a three-month period, while our study only considered the pre-weaning timeframe. There are known important functional differences between mice and pigs so it is unclear if the same outcome would be seen with SCID pigs at a later timepoint (Dawson et al., 2013; Meurens et al., 2012).

The lack of SCID genotype effect on the piglet gut microbiota that we observed could be due to the short timeframe in which fecal swabs were collected. We collected intestinal tissue at day one and day seven from some piglets in litter 33 and 34 that were not included in the microbiome analysis presented in this manuscript. These samples included tissues from four piglets at day one and three piglets at day seven, across all three genotypes. The tissues were prepared for histological analysis and there were no notable differences in the samples between the two timepoints as well as the different genotypes (data not shown). Lack of an effect by genotype was also seen in aspects of a 7-day study performed by Rajao and colleagues (2016) using this line of SCID pigs which analyzed the effect of influenza A on the immune system. While they observed notable differences of immune system function in some respects, when compared to non-SCID pigs in the same environment, there were parameters measured that did not appear to be different between the SCID group and the non-SCID group, such as IL-2 levels in the lungs (Rajao et al., 2016). The similarities between the two genotypes could have been due to the short time frame they were reporting on, as we report here. It could also be due to the

developmental stage of the innate immune system during this early period of neonatal development.

With the knowledge about the effect of the gut microbiota on the immune system, it could be hypothesized that non-SCID pigs, and SCID pigs raised in the same environment, would have similar immune development at early timepoints. Because the piglet bloodstream is not accessible to the maternal bloodstream as in humans, pigs are dependent on antibodies acquired at birth, otherwise they are highly susceptible to infection until they can create their own antibodies (Butler et al., 2009). The SCID and non-SCID piglets are raised and fed following the same protocol. Similar immune development in this time period could be due to the only antibodies they are acquiring is through the irradiated bovine colostrum they are fed. This could also be due to the lack of a complete gut microbiota to aid in the development of the host adaptive immune system that non-SCID pigs possess, unlike SCID pigs (Hill et al., 2012; Honda & Littman, 2016). Further studies performed for a longer duration of time are needed to determine if eventually there is an effect of SCID genotype on the gut microbiota of pigs. Regardless of the limitations present for these trials, the lack of a significant effect of SCID genotype on the piglet gut microbiota in our system is an important observation.

#### **4.2 Piglet age has a significant effect on the SCID piglet gut microbiota**

On a whole community scale, there were significant differences in bacterial microbiota composition between sampling timepoints, regardless of genotype. Litter 17 (2-strain) pairwise comparisons revealed a significant difference in day 2 samples compared to day 7-8, which is reflected in Figure 3A. More specifically, the relative abundance of uninoculated bacteria present at day 7-8 is much higher than the previous two timepoints as seen in Figure 4.

Litter 23 (6-strain inoculum) did not have any significant differences in microbiota in pairwise comparisons, after adjustments, due to lack of statistical power from the available samples (n=4), seen in Table 2. There was a higher relative abundance of bacteria, that were not inoculated, at day 14-15 when compared to the previous three timepoints. Litter 23 had a high relative abundance of other bacteria present at day 14-15 compared to the inoculated bacteria, this is also the timepoint that antibiotics were necessary for litter 17. The high relative abundance of other bacteria at the day 14-15 timepoint could be an indication of infection in the piglets.

Analysis for Litter 33 and 34 (7-strain inoculum), revealed the most consistency across the day 4, day 7-8, and day 14-15 timepoints, leading to the conclusion that the 7-strain is the most stable overtime in these piglets. When comparing relative abundance of the bacterial strains in the 6-strain and 7-strain defined microbiota over time, *Bacteroides* had the highest relative abundance for both groups by day 7-8. The relative abundance of the phylum *Bacteroidota* is second, behind *Firmicutes*, in the composition of gut microbiota in piglets (Wang et al., 2022), consistent with the high relative abundance we observed in our samples. The function of *Bacteroides* in the piglet gut is to degrade polysaccharides derived from ingested milk (Guevarra et al., 2019; Ye et al., 2021) and its relative abundance increases during the pre-weaning timeframe (Fukuda et al., 2021). This means *Bacteroides* can thrive in the gut microbiome of pre-weaned pigs due to milk intake. Inclusion of *Bacteroides* in the 6-strain and 7-strain defined microbiota, appears to have allowed the SCID piglet gut microbial composition to follow this trend.

Beta diversity statistics for litter 33 and 34 (Figure 3C) showed a significant difference between day 2 and day 14-15, as well as between day 4 and day 14-15. Alpha diversity (Supplementary Figure 2C) for observed species, richness, and diversity present a statistically significant difference between both pairwise comparisons. The only exception was for the alpha

diversity statistic of evenness as there was not a significant difference in evenness between day 4 and day 14-15. Overall, it appears an increase in the complexity and concentration of the inoculum, offers greater stability as piglets age. This was observed in previous research analyzing succession of the gut microbial community of pigs (Dong et al., 2023; Luo et al., 2022). Dong and colleagues (2023) determined the composition of the gut microbiota at an early timepoint varies between animals; however, as piglets get older, the composition of the gut microbiota was more similar across the group. This finding also supports the data Luo and colleagues (2022) reported, that there is a “core” microbiota, bacterial species consistently present, found in the gut of healthy piglets. Our findings support this published data which is a positive outcome for our results.

#### **4.3 Implications**

Pre-weaning was the time frame of focus for this study due to our concern of piglet loss from *C. difficile* or other rapid systemic infections. We did not see *C. difficile* infections while using any of the defined microbiota consortia tested, which suggests the inoculated bacteria is not allowing for the proliferation of *C. difficile*, if it was present. Additionally, the defined microbiotas did not cause harm to the piglets as none of the inoculated bacteria was cultured from the bloodstream of any piglets. Based on these results, we believe this is a promising initial step for the development of a defined microbiota, that can support the SCID pig gastrointestinal tract. The 7-strain inoculum is the most complex defined microbiota tested and it appears to offer increased stability to the piglet gut microbiota over the first two weeks of life, when compared to the 2-strain and 6-strain inoculum.

#### **4.4 Limitations**

More studies are necessary to examine the addition of a defined microbiota in SCID pigs as these biomedical models are important to gain insight in human processes. When working with a large immunodeficient biomedical animal model, there are many limitations present. The costs associated with creating and raising these litters is very high due to substantial labor input and lack of established husbandry protocols. Another challenge with SCID pigs is small litter sizes. The number of offspring in a SCID pig litter can be significantly smaller than a sow on a production farm which means there are less animals available for studies. This is, in part, the reason this study has a low sample size.

Creating a biocontainment room that can house SCID pigs safely is costly, and so is the effort required to keep these environments clean. Even though much effort was put towards keeping microorganisms out of the biocontainment facility during the piglet trials, it is difficult to keep the environment completely free of microorganisms that could be pathogenic to the SCID pigs. As seen in Figure 4, a portion of the overall microbiota present were bacteria that were not inoculated into the piglets. This leads to the conclusion that husbandry methods need to be improved to keep unwanted bacteria out of the piglets including environmental decontamination throughout the research study. There is also potential that the contamination present is from the sow and not from the environment, however, more research is needed in this area. Preventing the infiltration of pathogenic bacteria is difficult with a large animal model as care staff must enter the SCID pig biocontainment space to care for them. This makes it more challenging to keep an SPF environment when compared to smaller models, like SCID mice. The size of SCID mice allow them to live in small environments and they can be handled in a biosafety cabinet limiting potential for pathogen exposure, which is not possible for a large animal model.

Another challenge we encountered is the short sampling period. While we were focused on the pre-weaning time frame, that only allows 14-21 days for sample collection. We intended to continue collecting data after weaning, as well as giving a third dose of microbiota inoculum post weaning; however, medical intervention was necessary, hindering the use of data past the weaning timepoint. It is expected that the SCID piglet gut microbiota composition would shift after weaning, which is something to be analyzed in the future. Inoculating SCID piglets with a third or additional doses of microbiota after weaning or after antibiotic use would also be important to determine how different feeds or antibiotic use affects the inoculated bacteria.

Additionally, we used 16S rRNA gene amplicon sequencing. It is well known that amplicon sequencing is only semi-quantitative. The ideal method for greater accuracy when quantifying the amount of inoculated and uninoculated bacteria in the gut of the piglets is qPCR, of the extracted DNA, from the collected fecal swabs. By using amplicon sequencing, we were only able to present relative abundances of bacterial species (and not absolute abundances). We used DNA for sequencing, because of this we cannot prove that the obtained sequences were indeed from living organisms. Lastly, these were low biomass samples. To control for this in the sequencing process, we included many controls throughout the process. This included controls at the end of each DNA extraction kit using PCR grade water, blank fecal swabs, and PCR grade water at the end of the plate submitted for sequencing. These controls allowed us to account for possible contamination throughout the processing of samples. Then, for the analysis of samples, the decontam package, discussed in the materials and methods section of this manuscript, was used to detect contaminating DNA, and remove it. These methods were utilized to make our data the best quality they could be, despite their low biomass.

#### **4.5 Future studies**

In the future, a further increase in concentration of the inoculum or an addition of more bacterial strains to the inoculum could be implemented. These changes could lead to a decrease in the percent of uninoculated bacteria present in the SCID pigs. A more complex, but still defined, microbiota for use in gnotobiotic mice has been created (Cheng et al., 2022) which would be important to explore to improve the SCID pig model. The outcome of this mouse study was a gut microbiome that saw an increase in stability when challenged and displayed resistance against pathogenic *E. coli* (Cheng et al., 2022). As previously mentioned, a third (or additional) inoculation time point could be utilized post weaning or post antibiotics as both events lead to a change in the pig gut microbiota (Gao et al., 2018; Yu et al., 2021). With the changes to the SCID piglet husbandry protocol discussed in this section, the SCID pig could become a more reproducible and accessible model.

### **5 Conclusions**

This study looked at the effect of SCID genotype and piglet age on the defined microbiota in the gut of piglets. The results revealed there was no significant effect due to SCID genotype while piglet age had a significant effect on the gut microbiota of the piglets over time. More research is necessary to examine a defined microbiota in the husbandry of SCID piglets over a longer length of time, however, it appears the consortia we created were safe to administer to SCID piglets and may be beneficial in preventing *C. difficile*. The 7-strain defined microbiota, which had not only the greatest number of bacteria strains but also the highest concentration of bacteria. This appeared to offer more stability to the gut microbiota composition over time making it the most ideal candidate to give to SCID piglets. It will be added to our SCID pig



husbandry protocol allowing for rearing of SCID piglets to be more standardized, enabling reproducibility by other research groups.

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## Figure Legends

**Figure 1.** Principal coordinate analysis (PCoA) plot displays day 0 timepoint clustering with controls and environmental swabs leading to the exclusion of the day 0 timepoint from analysis. Distances between samples denote Bray-Curtis dissimilarity measures based on 16S rRNA gene amplicon sequencing. This plot shows that the composition of day 0 samples, taken immediately before inoculum was given, largely cluster with the control swabs, environmental swabs, and swabs from the mother's placenta. There was a significant difference between the non-day 0 swabs and the day 0 and control swabs ( $P=0.001$ ) that were detected using PERMANOVA. There was also a significant difference between Litter 17 and 23 swabs, labeled as "piglets\_17.23", compared to Litter 33 and 34, labeled as "piglets\_33.34", ( $P=0.001$ ) detected using PERMANOVA, so they were analyzed separately. Because of this, we removed the day 0 samples from the following analysis. More information about the controls and environmental swabs are provided in the materials and methods.

**Figure 2.** PCoA plots display no significant effect of SCID genotype on beta diversity of piglet gut microbiota when receiving the defined microbiota, regardless of piglet age. Based on 16S rRNA gene amplicon sequencing, distances between samples in PCoA plot denote Bray-Curtis dissimilarity measures. (A) Litter 17 received the 2-strain defined microbiota and there were significant differences due to genotype ( $P = 0.018$ ) detected using PERMANOVA. However, no statistical differences in pairwise comparisons were observed across genotypes. This litter received antibiotics prior to collection of day 14-15 swabs so they were omitted from the analysis. (B) Litter 23 received the 6-strain defined microbiota and significant differences due to genotype ( $P = 0.028$ ) were detected using PERMANOVA, however, unequal variance between groups was detected with PERMDISP2 ( $P = 0.009$ ). (C) Litters 33 and 34 received the 7-strain defined microbiota and no significant differences due to genotype ( $P=0.340$ ) were detected using PERMANOVA.

**Figure 3.** Significant effect of piglet age on beta diversity over time of gut microbiota of SCID piglets, regardless of genotype. Based on 16S rRNA gene amplicon sequencing, distances between samples in PCoA plot denote Bray-Curtis dissimilarity measures. (A) There were significant differences in the intestinal microbiota composition over time for litter 17, which received the 2-strain defined microbiota, due to piglet age ( $P = 0.001$ ) detected using PERMANOVA. This litter received antibiotics prior to the collection of day 14-15 swabs, so they were omitted from analysis. Pairwise comparison results can be seen in the results section. (B) Significant differences in microbiota composition over time for litter 23, which received the 6-strain defined microbiota, due to piglet age ( $P = 0.008$ ) were detected using PERMANOVA. Pairwise comparison results can be seen in the results section. (C) Significant differences in microbiota composition over time for litters 33 and 34, which received the 7-strain defined microbiota, due to piglet age ( $P = 0.001$ ) were detected using PERMANOVA. Pairwise comparison results can be seen in the results section.

**Figure 4.** Increase in inoculum complexity and concentration appears to offer greater stability to the gut microbiota of SCID piglets over time.

Based on 16S rRNA gene amplicon sequencing, this figure presents the relative abundance of each inoculated isolate when compared to the relative abundance of bacteria not administered in

the initial inoculum (“Other”- see Supplementary Table 1, 2, and 3, for the list of bacteria included in this category for each group). Litter 17 (2-strain inoculum) received antibiotics prior to the collection of day 14-15 swabs. Due to the potential effects of antibiotic administration on the microbiota these samples were excluded. It should be noted that the initial administered concentration of the 7-strain inoculum is higher than the 2-strain and 6-strain inocula. The fraction of “Other” bacteria remained relatively stable after day 2 in the piglets that received the 7-strain inoculum.

**Tables**

**Table 1.** Feeding timeline for cesarean-section derived SCID piglets

	Day 0	Day 1- before 36 hours	Day 1- after 36 hours	Day 2	Day 3	Day 4	Day 5	Day 6
Colostrum	100%	100%	50%	50%	50%	50%	50%	50%
Milk Replacer	0%	0%	50%	50%	50%	50%	50%	50%
	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Colostrum	25%	25%	25%	25%	25%	25%	25%	25%
Milk Replacer	75%	75%	75%	75%	75%	75%	75%	75%

Note: Bowls of colostrum and milk replacer were introduced to piglets at 12 hours of life.

**Table 2.** Microbiota treatment and genotype for each litter

	Genotype		Microbiota
Litter 17	A-SCID	n=2	2-strain
	G-SCID	n=2	
	Non-SCID	n=1	
Litter 23	A-SCID	n=2	6-strain
	Non-SCID	n=2	

**Table 3-2.** Continued

	Genotype		Microbiota
Litters 33 & 34	A-SCID	n=2	High concentration 7-strain
	G-SCID	n=5	
	Non-SCID	n=1	

Note: Artemis SCID denoted as A-SCID and IL2RG SCID denoted as G-SCID.

Table 3. Classification of each isolate in the inoculum from 16S rRNA gene sequencing

Inoculum			Isolate Name in Literature	SILVA Taxonomy (v138)	NCBI top match	Media	
7-strain	6-strain	2-strain	<i>novel</i>	<i>Peptoclostridium</i>	<i>Peptacetobacter spp.</i>	BHI + 1% YE	
			<i>Lactococcus lactis</i>	<i>Lactococcus</i>	<i>Lactococcus lactis</i>	MRS	
				<i>Lactococcus brevis</i> <sup>+</sup>	<i>Lactobacillus</i>	<i>Levilactobacillus brevis</i>	MRS
				<i>Bifidobacterium adolescentis</i> <sup>+</sup>	<i>Bifidobacterium</i>	<i>Bifidobacterium adolescentis</i>	MRS
				<i>Bifidobacterium longum</i> <sup>+</sup>	<i>Bifidobacterium</i>	<i>Bifidobacterium longum</i>	MRS
				<i>Clostridium clostridioforme</i> <sup>+</sup>	<i>Lachnoclostridium</i>	<i>Enterocloster clostridioformis</i>	BHI
			<i>Bacteroides vulgatus</i>	<i>Bacteroides</i>	<i>Phocaeicola vulgatus</i>	BHI	

<sup>+</sup>DOI: <https://doi.org/10.3920/BM2016.0225>

BHI = Brain Heart Infusion Agar; YE = Yeast Extract; MRS = Man – Rogosa – Sharpe

Note: All isolates, apart from *Clostridium sp.* TL22 and *Bacteroides vulgatus* were provided by the laboratory of Dr. Gireesh Rajashekara at The Ohio State University.

Table 4. Fecal swab collection timeline

Day 0 (Date of birth)	Day 1	Day 2	Day 4	Day 8	Day 15
Fecal swab collected prior to inoculation	<b>2<sup>nd</sup> microbiota inoculation</b>	Fecal swab collected	Fecal swab collected	Fecal swab collected	Fecal swab collected
<b>1<sup>st</sup> microbiota inoculation</b>					

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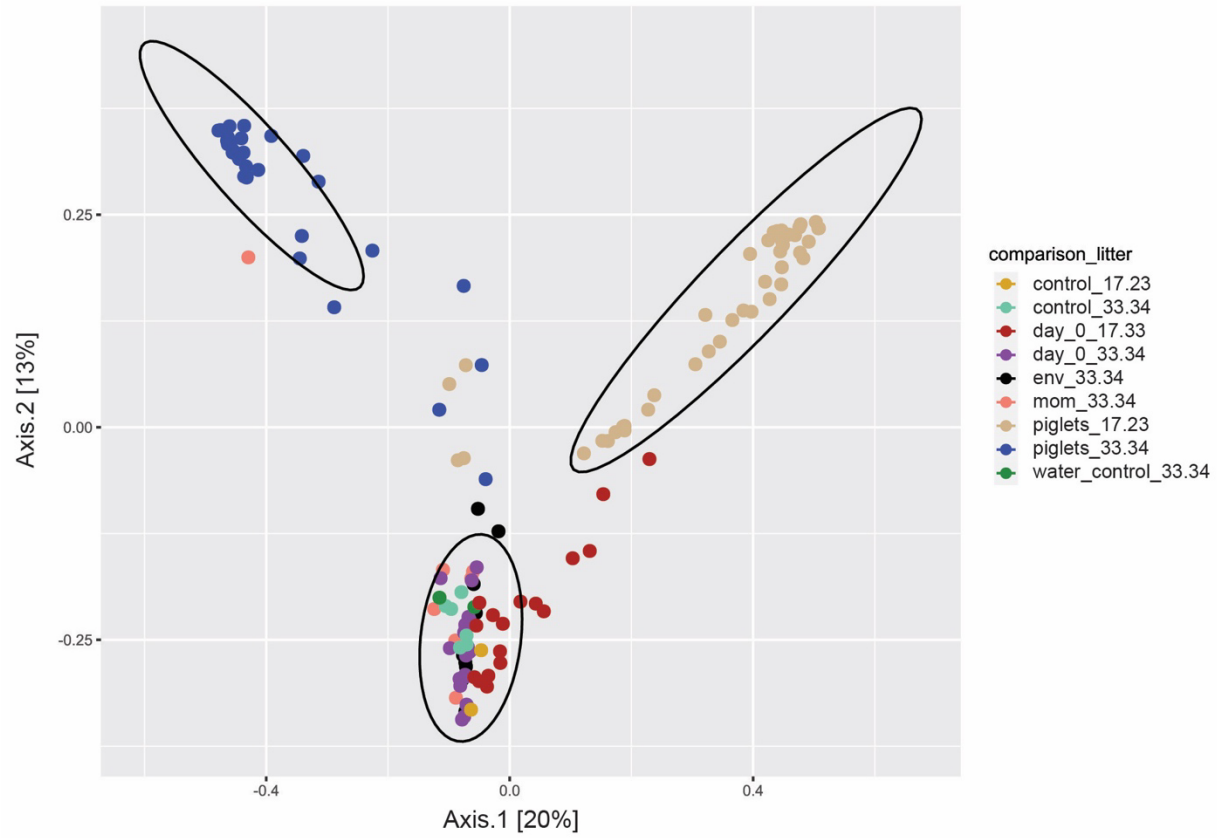
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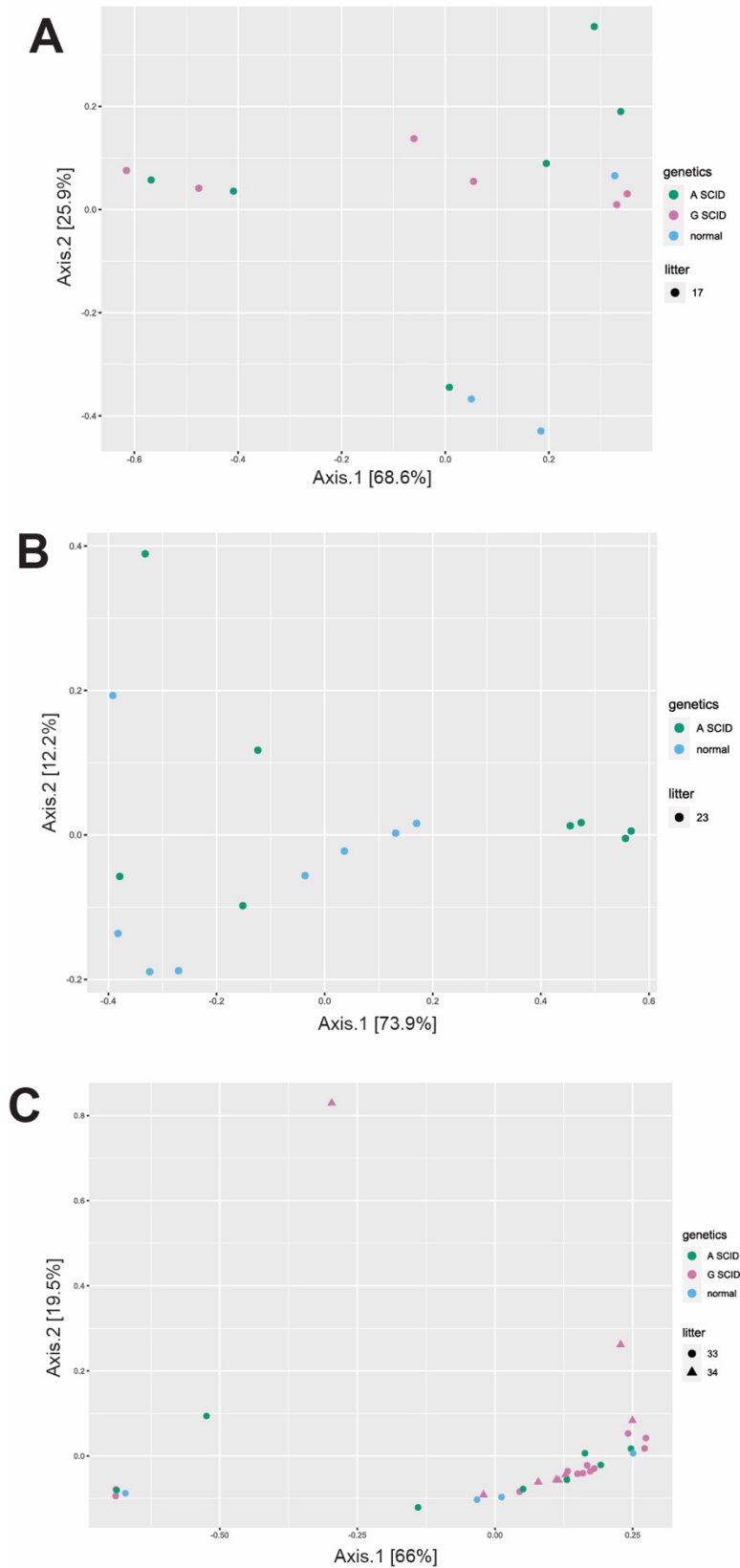
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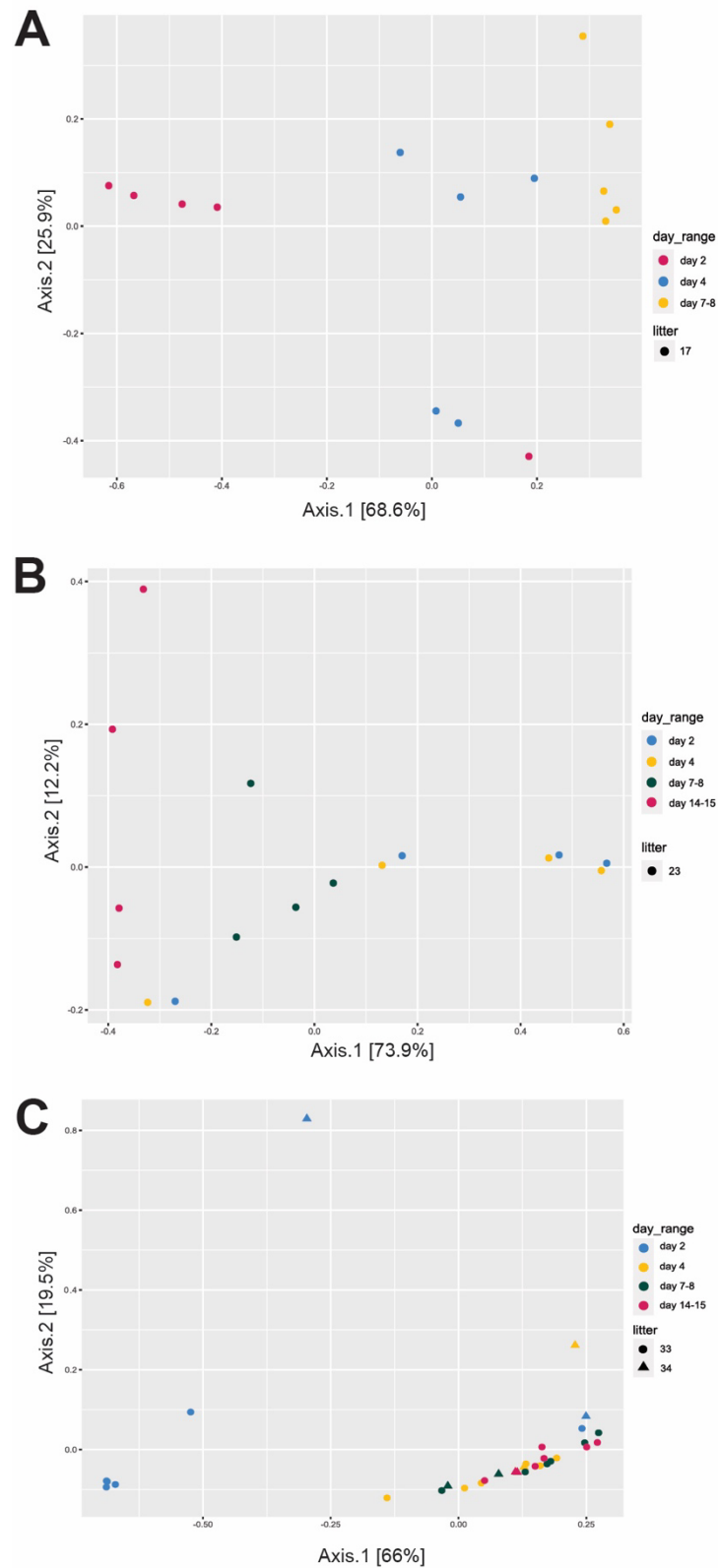
Figure 1



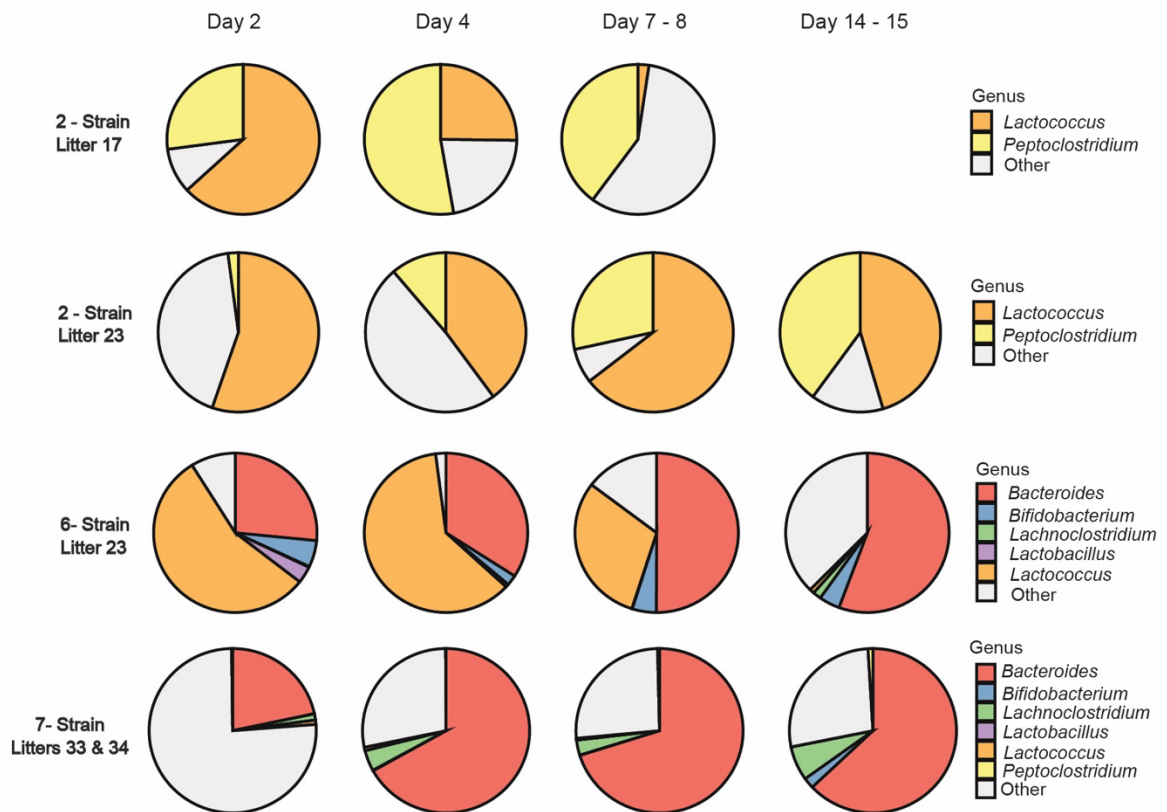
## Figure 2



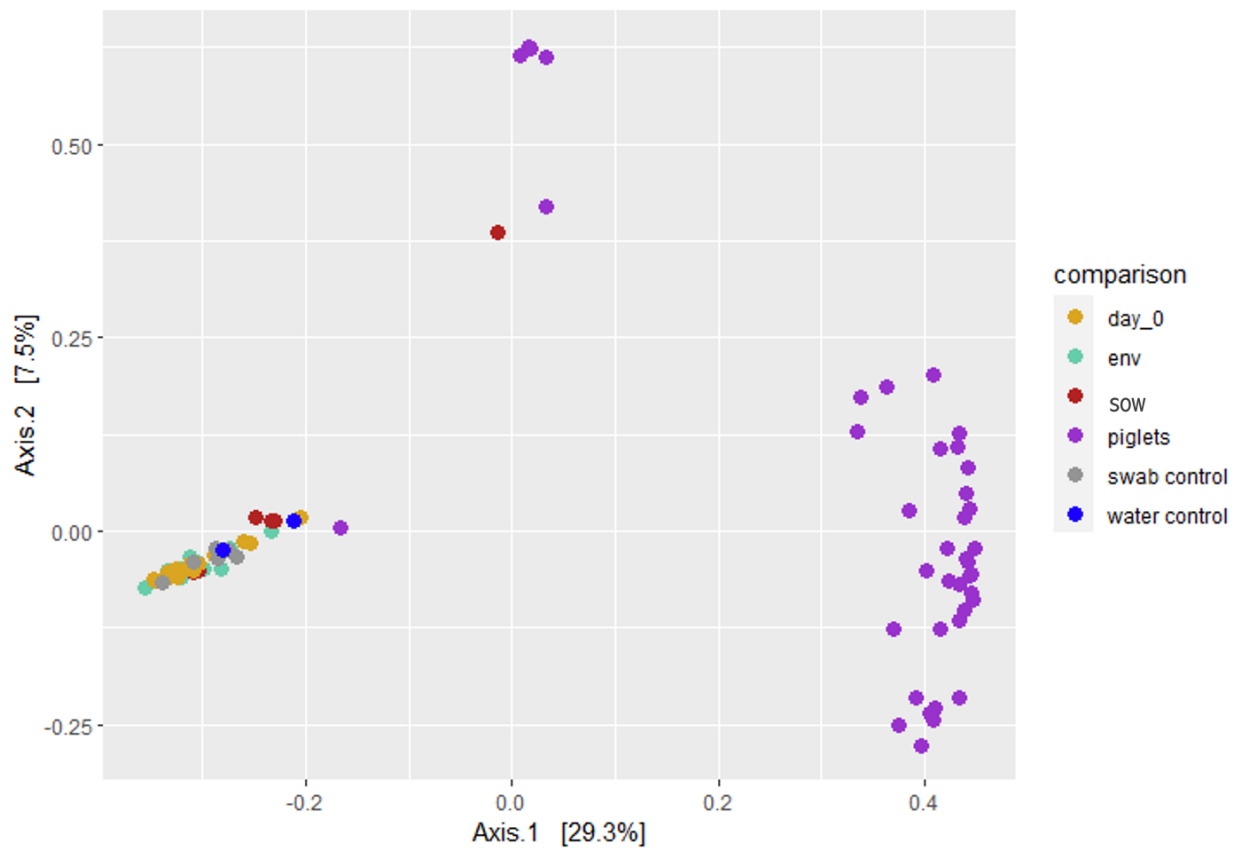
## Figure 3



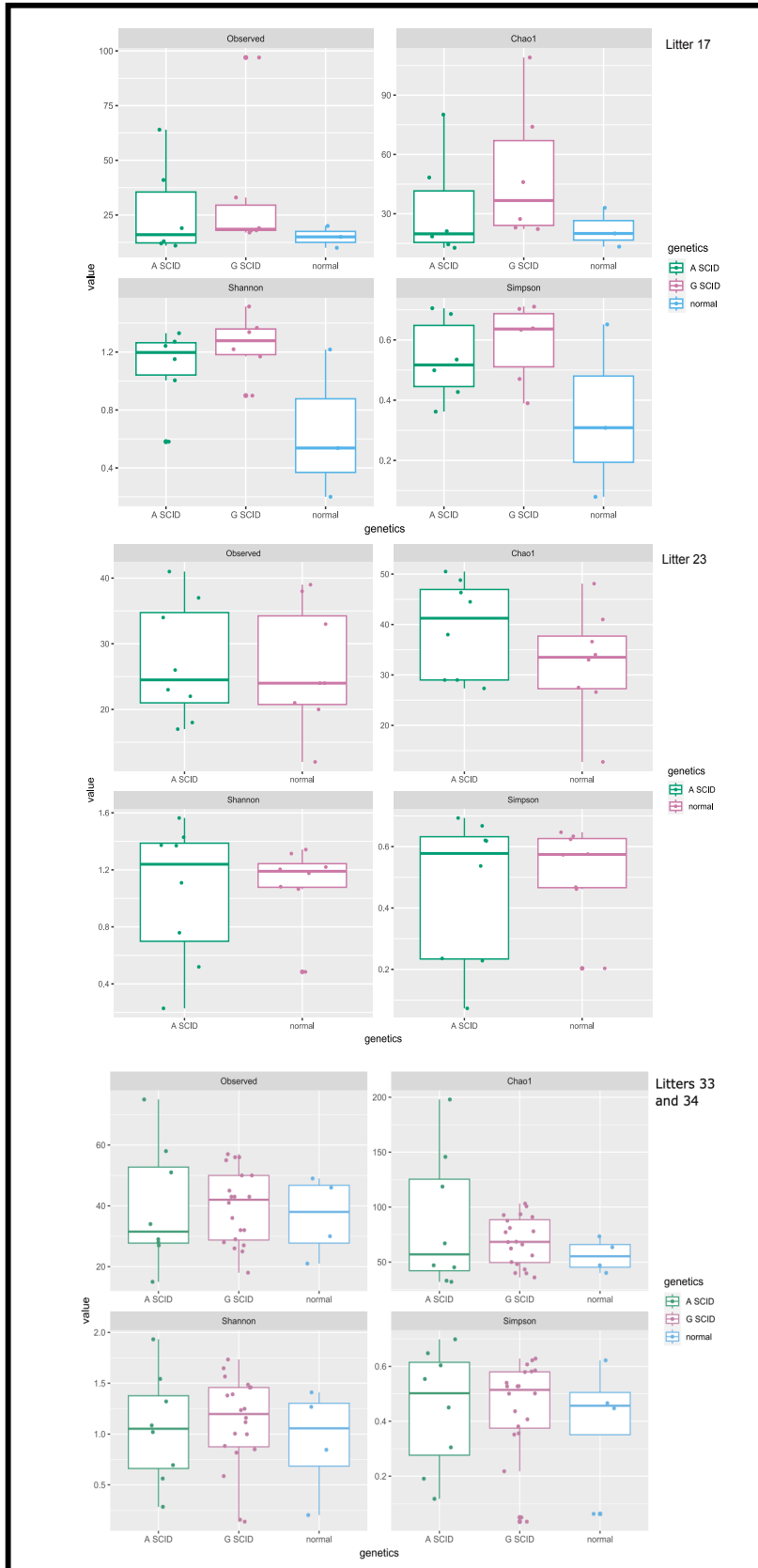
## Figure 4



Supplementary Figures and Tables



Supplementary Figure 1. Beta diversity of non-day 0 swabs are significantly different from the day 0 and control swabs for litters 33 and 34 and controls, depending on the decision to not include day 0 swabs in analysis. Due to the additional collection of multiple control samples from the cesarean section of Litter 33 and Litter 34 compared to the previous two trials involving Litter 17 and Litter 23, this PCoA plot was generated to further reinforce the decision to exclude the day 0 timepoint swabs. Distances between samples denote Bray-Curtis dissimilarity measures based on 16S rRNA gene amplicon sequencing. Swabs collected from every time point, other than day 0, were pooled together and labeled as “piglets” to determine the relationship between the day 0 swabs, control swabs and the rest of the time points. The “piglets” swabs are clearly clustered together, separate from the clustering of the rest of the swabs collected. This aids in the understanding that the swabs collected at day 0 are most like the control swabs, leading to the understanding that the Illumina MiSeq data from the day 0 samples is likely background noise and does not contain data that would need to be included in analysis.





Supplementary Figure 2. There was no significant effect of genotype on the alpha diversity of SCID piglet genotype on piglet gut microbiota, regardless of piglet age. This figure displays the number of observed species (Observed), species richness (Chao1), diversity (Shannon), and evenness (Simpson). **(A)** There were no significant differences ( $P>0.05$ ) in alpha diversity measures between the three genotypes in litter 17. **(B)** There were no significant differences ( $P>0.05$ ) in alpha diversity measures between the two genotypes in litter 23. **(C)** There were no significant differences ( $P>0.05$ ) in alpha diversity measures between the three genotypes in litter 33 and 34.



Supplementary Figure 3. There was a significant difference in alpha diversity of piglet gut microbiota due to piglet age, regardless of genotype. The figure compares the number of observed species (Observed), species richness (Chao1), diversity (Shannon), and evenness (Simpson) between the sampling timepoints. **(A)** Alpha diversity estimators for litter 17 revealed a significant difference in evenness ( $P = 0.026$ ) over time due to piglet age across the three timepoints. Pairwise comparisons for evenness revealed a significant difference ( $P=0.023$ ) between day 2 and day 7-8 in piglet microbiota due to piglet age. This litter received antibiotics prior to collection of day 14-15 swabs so they were omitted from analysis. **(B)** Alpha diversity estimators for litter 23 revealed a significant difference in observed species ( $P = 0.002$ ), species richness ( $P = 0.028$ ), and diversity ( $P = 0.014$ ) across the four timepoints due to piglet age. Pairwise comparisons show there was a significant difference in observed species between day 2 and day 4 ( $P=0.008$ ), between day 2 and day 7 ( $P=0.007$ ), and between day 4 and day 14 ( $P=0.019$ ). There was also a significant difference in diversity between day 4 and day 14 ( $P=0.010$ ). **(C)** Alpha diversity estimators for litters 33 and 34 revealed a significant difference in the number of observed species ( $P < 0.001$ ), species richness ( $P < 0.001$ ), diversity ( $P = 0.001$ ), and evenness ( $P = 0.017$ ) due to piglet age across the four timepoints due to piglet age. Pairwise comparisons show there was a significant difference in observed species between day 2 and day 7-8 ( $P < 0.001$ ), day 2 and day 14 ( $P < 0.001$ ), and day 4 and day 14 ( $P < 0.001$ ). There was a significant difference in species richness between day 2 and day 14 ( $P < 0.001$ ) and between day 4 and day 14 ( $P < 0.001$ ). There was a significant difference in diversity between day 2 and day 7-8 ( $P=0.029$ ), day 2 and day 14 ( $P=0.001$ ), and between day 4 and day 14 ( $P=0.036$ ). Lastly, there was a significant difference in evenness between day 2 and day 7-8 ( $P=0.048$ ) and between day 2 and day 14 ( $P=0.014$ ).

1  
2

Supplementary Table 1: List of “Other” bacteria as seen in Figure 4 for litter 17

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu00050	day 2	0.46280789	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria Incertae Sedis	Unknown_Family	Acidibacter
Otu00144	day 2	0.03100678	Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Aerococcaceae_unclassified
Otu00028	day 2	0.02561219	Verrucomicrobiota	Verrucomicrobiae	Verrucomicrobiales	Akkermansiaceae	Akkermansia
Otu00622	day 2	0.00131579	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Allorhizobium- Neorhizobium- Pararhizobium-Rhizobium
Otu00248	day 2	0.06239042	Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamonadaceae	Aquabacterium
Otu00186	day 2	0.05088284	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	Asinibacterium
Otu01073	day 2	0.00159885	Firmicutes	Bacilli	Bacilli_unclassified	Bacilli_unclassified	Bacilli_unclassified
Otu00305	day 2	0.00162946	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus
Otu00003	day 2	0.03915265	Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides
Otu01214	day 2	0.01622078	Actinobacteriota	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacteriaceae_ unclassified
Otu01069	day 2	0.03179259	Actinobacteriota	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium
Otu00008	day 2	0.04868726	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Blautia
Otu00998	day 2	0.00720629	Actinobacteriota	Actinobacteria	Micrococcales	Dermabacteraceae	Brachybacterium
Otu00931	day 2	0.01805883	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Bradyrhizobium
Otu00193	day 2	0.07756194	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Carnobacterium
Otu00364	day 2	0.03124252	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	Chitinophagaceae_ unclassified
Otu00351	day 2	0.032511	Cyanobacteria	Cyanobacteriia	Chloroplast	Chloroplast_fa	Chloroplast_ge
Otu01037	day 2	0.04025832	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	Christensenellaceae_R- 7_group
Otu00571	day 2	0.01279079	Bacteroidota	Bacteroidia	Flavobacteriales	Weeksellaceae	Cloacibacterium
Otu00055	day 2	0.03037813	Firmicutes	Clostridia	Clostridia_UCG-014	Clostridia_UCG-014_fa	Clostridia_UCG-014_ge
Otu00015	day 2	0.00590786	Firmicutes	Clostridia	Peptostreptococcales- Tissierellales	Peptostreptococcaceae	Clostridioides
Otu00012	day 2	0.24767855	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium_sensu_stricto_ 1

Table 1: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu00035	day 2	0.0031977	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium_sensu_stricto_13
Otu00096	day 2	0.0031977	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	Colidextribacter
Otu00039	day 2	0.07864187	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Coprococcus
Otu00198	day 2	0.07857934	Actinobacteriota	Actinobacteria	Corynebacteriales	Corynebacteriaceae	Corynebacterium
Otu00021	day 2	0.0047498	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Eisenbergiella
Otu00766	day 2	0.00291464	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacterales_unclassified	Enterobacterales_unclassified
Otu00414	day 2	0.03859076	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Enterobacteriaceae_unclassified
Otu00139	day 2	0.02933029	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcaceae_unclassified
Otu00004	day 2	0.83528794	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus
Otu00071	day 2	0.08445593	Firmicutes	Bacilli	Erysipelotrichales	Erysipelatoclostridiaceae	Erysipelatoclostridium
Otu00032	day 2	0.01166343	Firmicutes	Bacilli	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_ge
Otu00005	day 2	0.11720355	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Escherichia-Shigella
Otu00308	day 2	0.02718043	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcales-Tissierellales_fa	Finegoldia
Otu00022	day 2	0.04739935	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	Flavonifractor
Otu00125	day 2	0.01845173	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Fusicatenibacter
Otu01097	day 2	0.0031977	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Granulicatella
Otu00117	day 2	0.29460523	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Halomonas
Otu00440	day 2	0.00959309	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Hyphomicrobiaceae_unclassified
Otu00031	day 2	0.04636662	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Intestinibacter
Otu00302	day 2	0.03360644	Actinobacteriota	Actinobacteria	Micrococcales	Micrococcaceae	Kocuria
Otu00207	day 2	0.00325892	Firmicutes	Bacilli	Bacillales	Planococcaceae	Kurthia
Otu00019	day 2	0.0031977	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnoclostridium
Otu00041	day 2	0.05743266	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae_ND3007_group

Table 1: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu00132	day 2	0.00162946	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae_NK4A136 group
Otu00062	day 2	0.07354705	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae_UCG-004
Otu00013	day 2	0.32995069	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae_unclassified
Otu01143	day 2	0.01309679	Firmicutes	Bacilli	Lactobacillales	Lactobacillales_unclassified	Lactobacillales_unclassified
Otu01652	day 2	0.01923986	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
Otu01300	day 2	0.0063954	Actinobacteriota	Actinobacteria	Micrococcales	Microbacteriaceae	Leifsonia
Otu00206	day 2	0.09818715	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Leuconostoc
Otu00460	day 2	0.0063954	Firmicutes	Bacilli	Lactobacillales	Listeriaceae	Listeria
Otu01220	day 2	0.01610315	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	Macrococcus
Otu00228	day 2	0.00263158	Vertebrata	Mammalia	Mammalia_or	Mammalia_fa	Mammalia_ge
Otu00014	day 2	0.11296793	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Marvinbryantia
Otu00053	day 2	0.5965127	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Mesorhizobium
Otu01208	day 2	0.00479655	Proteobacteria	Gammaproteobacteria	Burkholderiales	Methylophilaceae	Methylobacillus
Otu00354	day 2	0.07354705	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylobacterium-Methylobacterium
Otu00278	day 2	0.07380693	Actinobacteriota	Actinobacteria	Micrococcales	Micrococcaceae	Micrococcus
Otu00038	day 2	0.04316892	Firmicutes	Clostridia	Monoglobales	Monoglobaceae	Monoglobus
Otu01270	day 2	0.02877928	Bacteroidota	Bacteroidia	Bacteroidales	Muribaculaceae	Muribaculaceae_ge
Otu01386	day 2	0.02516145	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	NS5_marine_group
Otu00221	day 2	0.00159885	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Negativicoccus
Otu00320	day 2	0.07034935	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Ochrobactrum
Otu00204	day 2	0.00479655	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	Oscillibacter
Otu00056	day 2	0.00283728	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	Oscillospira
Otu00099	day 2	0.00786835	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	Oscillospiraceae_unclassified
Otu01140	day 2	0.00799424	Firmicutes	Clostridia	Oscillospirales	Oscillospirales_unclassified	Oscillospirales_unclassified

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Table 1: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu00353	day 2	0.03155685	Myxococcota	Polyangia	Polyangiales	Polyangiaceae	Pajaroellobacter
Otu00174	day 2	0.03221272	Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamonadaceae	Paucibacter
Otu00018	day 2	0.01973626	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Pediococcus
Otu00316	day 2	0.00620417	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcales-Tissierellales fa	Peptoniphilus
Otu00077	day 2	0.12474148	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Peptostreptococcaceae_unclassified
Otu00307	day 2	0.03197698	Firmicutes	Bacilli	Bacillales	Planococcaceae	Planococcaceae_unclassified
Otu00902	day 2	0.04476777	Proteobacteria	Gammaproteobacteria	Alteromonadales	Pseudoalteromonadaceae	Pseudoalteromonas
Otu00149	day 2	0.03004775	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
Otu00194	day 2	0.03520529	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	Puia
Otu00742	day 2	0.0167743	Firmicutes	Bacilli	RF39	RF39_fa	RF39_ge
Otu00725	day 2	0.01279079	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Ralstonia
Otu01537	day 2	0.0058905	Actinobacteriota	Actinobacteria	Corynebacteriales	Nocardiaceae	Rhodococcus
Otu00059	day 2	0.00498432	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Romboutsia
Otu01164	day 2	0.00294525	Actinobacteriota	Actinobacteria	Micrococcales	Micrococcaceae	Rothia
Otu00057	day 2	0.02773172	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Ruminococcaceae_ge
Otu00110	day 2	0.0031977	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Ruminococcaceae_unclassified
Otu00024	day 2	0.16731705	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Ruminococcus
Otu00061	day 2	0.48799764	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium
Otu00225	day 2	0.11047773	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Selenomonadaceae	Selenomonas
Otu01390	day 2	0.02476855	Bacteroidota	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	Sphingobacterium
Otu00160	day 2	0.03042461	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas
Otu00010	day 2	0.14952409	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	Staphylococcus
Otu00001	day 2	3.12373544	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
Otu00037	day 2	0.01174201	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Subdoligranulum
Otu00011	day 2	0.13775386	Firmicutes	Bacilli	Erysipelotrichales	Erysipelotrichaceae	Turicibacter

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Table 1: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu00030	day 2	0.15332924	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	UCG-005
Otu00044	day 2	0.06091342	Firmicutes	Clostridia	Oscillospirales	Butyricocccaceae	UCG-008
Otu00823	day 2	0.0623551	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria	Unknown_Family	Unknown_Family_ge
Otu01179	day 2	0.02118409	Firmicutes	Bacilli	Lactobacillales	Vagococcaceae	Vagococcus
Otu00058	day 2	0.00167743	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Weissella
Otu01601	day 2	0.01282206	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Yersiniaceae	Yersiniaceae_unclassified
Otu00050	day 4	0.00139519	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria	Unknown_Family	Acidibacter
Otu00115	day 4	0.00114653	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Anaerotruncus
Otu00768	day 4	0.00121492	Actinobacteriota	Actinobacteria	Actinomycetales	Actinomycetaceae	Arcanobacterium
Otu01073	day 4	0.03155229	Firmicutes	Bacilli	Bacilli_unclassified	Bacilli_unclassified	Bacilli_unclassified
Otu00003	day 4	0.01063846	Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides
Otu01069	day 4	0.00377049	Actinobacteriota	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium
Otu00008	day 4	0.01405289	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Blautia
Otu00193	day 4	0.0034635	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Carnobacterium
Otu00055	day 4	0.00121492	Firmicutes	Clostridia	Clostridia_UCG-014	Clostridia_UCG-014_fa	Clostridia_UCG-014_ge
Otu01227	day 4	0.00139519	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridiaceae_unclassified
Otu00015	day 4	0.00224858	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Clostridioides
Otu00012	day 4	8.83884999	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium_sensu_stricto_1
Otu00096	day 4	0.00112429	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	Colidextribacter
Otu00039	day 4	0.00121492	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Coprococcus
Otu00766	day 4	0.00139519	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriales_unclassified	Enterobacteriales_unclassified
Otu00414	day 4	0.00229305	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacteriaceae_unclassified
Otu00004	day 4	0.02308148	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus
Otu00005	day 4	0.0108639	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Escherichia-Shigella



Table 1: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu00022	day 4	0.00114653	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	Flavonifractor
Otu00105	day 4	0.00139519	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Hungatella
Otu00031	day 4	0.00114653	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Intestinibacter
Otu00062	day 4	0.00261011	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae_UCG-004
Otu00013	day 4	0.0115815	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae_unclassified
Otu01143	day 4	0.00357636	Firmicutes	Bacilli	Lactobacillales	Lactobacillales_unclassified	Lactobacillales_unclassified
Otu00206	day 4	0.00242984	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Leuconostoc
Otu00014	day 4	0.00734686	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Marvinbryantia
Otu00053	day 4	0.00114653	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Mesorhizobium
Otu00038	day 4	0.00382503	Firmicutes	Clostridia	Monoglobales	Monoglobaceae	Monoglobus
Otu00056	day 4	0.00112429	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	Oscillospira
Otu00353	day 4	0.00121492	Myxococcota	Polyangia	Polyangiales	Polyangiaceae	Pajaroellobacter
Otu00077	day 4	0.00242984	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Peptostreptococcaceae_unclassified
Otu00194	day 4	0.00233921	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	Puia
Otu00286	day 4	0.00121492	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Roseburia
Otu00057	day 4	0.00121492	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Ruminococcaceae_ge
Otu00024	day 4	0.00242984	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Ruminococcus
Otu00061	day 4	0.00114653	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium
Otu00010	day 4	0.00114653	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	Staphylococcus
Otu00001	day 4	0.06600484	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
Otu00011	day 4	12.9688387	Firmicutes	Bacilli	Erysipelotrichales	Erysipelotrichaceae	Turicibacter
Otu00030	day 4	0.00121492	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	UCG-005
Otu00034	day 7-8	0.0013758	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Anaerostipes
Otu01073	day 7-8	0.00444545	Firmicutes	Bacilli	Bacilli_unclassified	Bacilli_unclassified	Bacilli_unclassified
Otu00003	day 7-8	0.0027645	Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides

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Table 1: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu01069	day 7-8	0.0013887	Actinobacteriota	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium
Otu00008	day 7-8	0.00861595	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Blautia
Otu00193	day 7-8	0.00333611	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Carnobacterium
Otu01227	day 7-8	0.02878946	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridiaceae_unclassified
Otu00015	day 7-8	0.0017234	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Clostridioides
Otu00012	day 7-8	39.1987659	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium_sensu_stricto_1
Otu00035	day 7-8	0.0017234	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium_sensu_stricto_13
Otu00414	day 7-8	0.00305675	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacteriaceae_unclassified
Otu00004	day 7-8	0.02739467	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus
Otu00005	day 7-8	0.01826179	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Escherichia-Shigella
Otu00022	day 7-8	0.00344679	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	Flavonifractor
Otu00041	day 7-8	0.0017234	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae_ND3007_group
Otu00013	day 7-8	0.00277739	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae_unclassified
Otu00014	day 7-8	0.00309985	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Marvinbryantia
Otu00038	day 7-8	0.0013758	Firmicutes	Clostridia	Monoglobales	Monoglobaceae	Monoglobus
Otu00056	day 7-8	0.0017234	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	Oscillospira
Otu00174	day 7-8	0.0013887	Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamonadaceae	Paucibacter
Otu00018	day 7-8	0.0013758	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Pediococcus
Otu00316	day 7-8	0.0013758	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcales-Tissierellales_fa	Peptoniphilus
Otu00061	day 7-8	0.0013887	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium
Otu00010	day 7-8	0.27221598	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	Staphylococcus
Otu00001	day 7-8	0.05486272	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
Otu00064	day 7-8	0.0013758	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Terrisporobacter
Otu00011	day 7-8	18.1295372	Firmicutes	Bacilli	Erysipelotrichales	Erysipelotrichaceae	Turicibacter

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Supplementary Table 2: List of “Other” bacteria as seen in Figure 4 for litter 23

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu01561	day 2	0.023978	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter
Otu00247	day 2	0.00945	Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Aerococcaceae_unclassified
Otu00908	day 2	0.002363	Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Aerococcus
Otu01059	day 2	0.115113	Proteobacteria	Gammaproteobacteria	Burkholderiales	Alcaligenaceae	Alcaligenaceae_unclassified
Otu00287	day 2	0.065779	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Alishewanella
Otu00297	day 2	0.008041	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Alloprevotella
Otu00186	day 2	0.008041	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	Asinibacterium
Otu01334	day 2	0.034877	Actinobacteriota	Coriobacteriia	Coriobacteriales	Atopobiaceae	Atopobiaceae_unclassified
Otu00306	day 2	0.00402	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus
Otu01214	day 2	0.004725	Actinobacteriota	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacteriaceae_unclassified
Otu00020	day 2	0.008745	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Blautia
Otu00193	day 2	0.110413	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Carnobacterium
Otu00350	day 2	0.00201	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	Chitinophagaceae_unclassified
Otu00080	day 2	0.344455	Cyanobacteria	Cyanobacteriia	Chloroplast	Chloroplast_fa	Chloroplast_ge
Otu00012	day 2	0.034537	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium_sensu_stricto_1
Otu00039	day 2	0.00201	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Coprococcus
Otu00198	day 2	0.058019	Actinobacteriota	Actinobacteria	Corynebacteriales	Corynebacteriaceae	Corynebacterium
Otu00285	day 2	0.002363	Deinococcota	Deinococci	Deinococcales	Deinococcaceae	Deinococcus
Otu00021	day 2	0.00201	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Eisenbergiella
Otu00637	day 2	0.016445	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Ellin6055
Otu00007	day 2	0.00201	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacteriaceae_unclassified
Otu00004	day 2	0.101352	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus
Otu00005	day 2	0.09375	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Escherichia-Shigella
Otu00192	day 2	0.102033	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Fructobacillus
Otu00663	day 2	0.028778	Fusobacteriota	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium

Table 2: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu00117	day 2	0.296749	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Halomonas
Otu00031	day 2	0.004111	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Intestinibacter
Otu01648	day 2	0.017438	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	Jeotgalicoccus
Otu00132	day 2	0.00201	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae_NK4A136_group
Otu00372	day 2	0.255415	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae_unclassified
Otu00224	day 2	0.002363	Firmicutes	Bacilli	Lactobacillales	Lactobacillales_unclassified	Lactobacillales_unclassified
Otu00075	day 2	0.203269	Fusobacteriota	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichiaceae_unclassified
Otu00206	day 2	0.13208	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Leuconostoc
Otu01220	day 2	0.006735	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	Macrococcus
Otu00014	day 2	0.00402	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Marvinbryantia
Otu00349	day 2	0.023978	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobrevibacter
Otu01208	day 2	0.014776	Proteobacteria	Gammaproteobacteria	Burkholderiales	Methylophilaceae	Methylobacillus
Otu01284	day 2	0.074001	Firmicutes	Clostridia	Oscillospirales	Oscillospirales_unclassified	Oscillospirales_unclassified
Otu00353	day 2	0.020556	Myxococcota	Polyangia	Polyangiales	Polyangiaceae	Pajaroellobacter
Otu00960	day 2	0.143891	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Pasteurella
Otu00946	day 2	0.148002	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Pasteurellaceae_unclassified
Otu00006	day 2	0.036913	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Peptoclostridium
Otu00077	day 2	0.211406	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Peptostreptococcaceae_unclassified
Otu00826	day 2	0.008719	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcales-Tissierellales_fa	Peptostreptococcales-Tissierellales_fa_unclassified
Otu00232	day 2	0.014175	Firmicutes	Bacilli	Bacillales	Planococcaceae	Planococcaceae_unclassified
Otu00265	day 2	0.169172	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
Otu00902	day 2	0.014071	Proteobacteria	Gammaproteobacteria	Alteromonadales	Pseudoalteromonadaceae	Pseudoalteromonas

Table 2: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu00149	day 2	0.18263	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
Otu00310	day 2	0.024667	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Ralstonia
Otu01189	day 2	0.023978	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rhodobacteraceae_unclassified
Otu01537	day 2	0.011264	Actinobacteriota	Actinobacteria	Corynebacteriales	Nocardiaceae	Rhodococcus
Otu01683	day 2	0.023625	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	Salinicoccus
Otu00222	day 2	0.004111	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Salinicola
Otu00266	day 2	0.026941	Firmicutes	Bacilli	Bacillales	Planococcaceae	Solibacillus
Otu00214	day 2	0.020817	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas
Otu00612	day 2	0.065779	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	Staphylococcaceae_unclassified
Otu00010	day 2	0.237393	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	Staphylococcus
Otu00016	day 2	4.860796	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
Otu00997	day 2	0.00436	Deinococcota	Deinococci	Thermales	Thermaceae	Thermus
Otu00541	day 2	0.00402	Spirochaetota	Spirochaetia	Spirochaetales	Spirochaetaceae	Treponema
Otu00011	day 2	0.060981	Firmicutes	Bacilli	Erysipelotrichales	Erysipelotrichaceae	Turicibacter
Otu00869	day 2	0.172669	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	UCG-002
Otu00026	day 2	0.00218	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	UCG-005
Otu00823	day 2	0.008041	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria Incertae Sedis	Unknown_Family	Unknown_Family_ge
Otu00587	day 2	0.00218	Firmicutes	Negativicutes	Veillonellales- Selenomonadales	Veillonellaceae	Veillonella
Otu01598	day 2	0.023978	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio
Otu01311	day 2	0.021289	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Weissella
Otu01126	day 2	0.098668	Actinobacteriota	Acidimicrobiia	Microtrichales	Microtrichaceae	uncultured
Otu00770	day 2	0.00218	Acidobacteriota	Vicinamibacteria	Vicinamibacterales	uncultured	uncultured_ge
Otu01561	day 4	0.006866	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter
Otu00768	day 4	0.002289	Actinobacteriota	Actinobacteria	Actinomycetales	Actinomycetaceae	Arcanobacterium
Otu00233	day 4	0.001749	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Aureimonas
Otu00020	day 4	0.004038	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Blautia

Table 2: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu00193	day 4	0.050102	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Carnobacterium
Otu00080	day 4	0.003498	Cyanobacteria	Cyanobacteria	Chloroplast	Chloroplast_fa	Chloroplast_ge
Otu00508	day 4	0.001749	Actinobacteriota	Actinobacteria	Micrococcales	Micrococcaceae	Citricoccus
Otu00012	day 4	0.006155	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium_sensu_stricto_1
Otu00021	day 4	0.004406	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Eisenbergiella
Otu00007	day 4	0.002951	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacteriaceae_unclassified
Otu00004	day 4	0.014257	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus
Otu00032	day 4	0.002289	Firmicutes	Bacilli	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_ge
Otu00005	day 4	0.014599	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Escherichia-Shigella
Otu00192	day 4	0.064482	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Fructobacillus
Otu00125	day 4	0.002289	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Fusicatenibacter
Otu00041	day 4	0.004038	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae_ND3007_group
Otu00372	day 4	0.01093	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae_unclassified
Otu00224	day 4	0.001749	Firmicutes	Bacilli	Lactobacillales	Lactobacillales_unclassified	Lactobacillales_unclassified
Otu00206	day 4	0.046391	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Leuconostoc
Otu00038	day 4	0.002289	Firmicutes	Clostridia	Monoglobales	Monoglobaceae	Monoglobus
Otu00056	day 4	0.001749	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	Oscillospira
Otu00006	day 4	0.005068	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Peptoclostridium
Otu00077	day 4	0.019777	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Peptostreptococcaceae_unclassified
Otu00149	day 4	0.005901	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
Otu00310	day 4	0.001749	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Ralstonia
Otu01537	day 4	0.001749	Actinobacteriota	Actinobacteria	Corynebacteriales	Nocardiaceae	Rhodococcus
Otu00195	day 4	0.002289	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Ruminococcaceae_unclassified
Otu00225	day 4	0.003498	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Selenomonadaceae	Selenomonas

Table 2: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu00010	day 4	0.045983	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	Staphylococcus
Otu00016	day 4	1.67786	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
Otu00011	day 4	0.01231	Firmicutes	Bacilli	Erysipelotrichales	Erysipelotrichaceae	Turicibacter
Otu00026	day 4	0.001749	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	UCG-005
Otu01601	day 4	0.002951	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Yersiniaceae	Yersiniaceae_unclassified
Otu00020	day 7-8	0.006292	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Blautia
Otu00193	day 7-8	0.028755	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Carnobacterium
Otu00081	day 7-8	0.004256	Firmicutes	Clostridia	Clostridia_UCG-014	Clostridia_UCG-014_fa	Clostridia_UCG-014_ge
Otu00012	day 7-8	0.016881	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium_sensu_stricto_1
Otu00035	day 7-8	0.001929	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium_sensu_stricto_13
Otu00021	day 7-8	0.001929	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Eisenbergiella
Otu00007	day 7-8	0.001729	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacteriaceae_unclassified
Otu00004	day 7-8	0.031046	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus
Otu00005	day 7-8	0.020392	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Escherichia-Shigella
Otu00192	day 7-8	0.027243	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Fructobacillus
Otu00105	day 7-8	0.001729	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Hungatella
Otu00062	day 7-8	0.001929	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae_UCG-004
Otu00372	day 7-8	0.011573	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae_unclassified
Otu00075	day 7-8	0.002526	Fusobacteriota	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichiaceae_unclassified
Otu00206	day 7-8	0.039716	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Leuconostoc
Otu00014	day 7-8	0.003659	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Marvinbryantia
Otu00038	day 7-8	0.001929	Firmicutes	Clostridia	Monoglobales	Monoglobaceae	Monoglobus
Otu00018	day 7-8	0.001929	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Pediococcus
Otu00006	day 7-8	0.008115	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Peptoclostridium
Otu00310	day 7-8	0.002181	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Ralstonia

Table 2: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu00059	day 7-8	0.047932	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Romboutsia
Otu00612	day 7-8	0.001929	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	Staphylococcaceae_unclassified
Otu00010	day 7-8	12.49783	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	Staphylococcus
Otu00016	day 7-8	1.910357	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
Otu00011	day 7-8	0.009751	Firmicutes	Bacilli	Erysipelotrichales	Erysipelotrichaceae	Turicibacter
Otu00026	day 7-8	0.002181	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	UCG-005
Otu00908	day 14-15	0.059848	Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Aerococcus
Otu00034	day 14-15	0.001418	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Anaerostipes
Otu00589	day 14-15	0.063017	Firmicutes	Bacilli	Bacilli_unclassified	Bacilli_unclassified	Bacilli_unclassified
Otu00020	day 14-15	0.081081	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Blautia
Otu00193	day 14-15	0.014242	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Carnobacterium
Otu00091	day 14-15	0.253472	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridiaceae_unclassified
Otu00015	day 14-15	8.604709	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Clostridioides
Otu00012	day 14-15	0.017314	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium_sensu_stricto_1
Otu00150	day 14-15	0.001418	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	DTU089
Otu00007	day 14-15	0.002836	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacteriaceae_unclassified
Otu00139	day 14-15	0.019506	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcaceae_unclassified
Otu00004	day 14-15	18.72933	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus
Otu00005	day 14-15	0.022649	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Escherichia-Shigella
Otu00022	day 14-15	0.279837	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	Flavonifractor
Otu00192	day 14-15	0.018393	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Fructobacillus



Table 2: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu00372	day 14-15	0.127806	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae_unclassified
Otu00224	day 14-15	0.13169	Firmicutes	Bacilli	Lactobacillales	Lactobacillales_unclassified	Lactobacillales_unclassified
Otu00206	day 14-15	0.026631	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Leuconostoc
Otu00018	day 14-15	0.001957	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Pediococcus
Otu00006	day 14-15	0.013163	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Peptoclostridium
Otu00077	day 14-15	0.021369	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Peptostreptococcaceae_unclassified
Otu00059	day 14-15	1.572609	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Romboutsia
Otu00060	day 14-15	0.001957	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Ruminococcus
Otu00612	day 14-15	0.001418	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	Staphylococcaceae_unclassified
Otu00010	day 14-15	6.106252	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	Staphylococcus
Otu00016	day 14-15	1.444683	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
Otu00011	day 14-15	0.015592	Firmicutes	Bacilli	Erysipelotrichales	Erysipelotrichaceae	Turicibacter
Otu00026	day 14-15	0.001418	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	UCG-005

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Supplementary Table 3: List of “Other” bacteria as seen in Figure 4 for litters 33 and 34

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu02660	day 2	0.002034	Bdellovibrionota	Oligoflexia	0319-6G20	0319-6G20_fa	0319-6G20_ge
Otu01343	day 2	0.000151	Chloroflexi	Ktedonobacteria	Ktedonobacterales	Ktedonobacteraceae	1959-1
Otu03009	day 2	0.001316	Firmicutes	Bacilli	Acholeplasmatales	Acholeplasmataceae	Acholeplasmataceae_unclassified
Otu01091	day 2	0.000105	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria _Incertae Sedis	Unknown_Family	Acidibacter
Otu00052	day 2	0.001368	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter
Otu00300	day 2	0.001618	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Actinobacillus
Otu00284	day 2	0.003138	Proteobacteria	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	Aeromonas
Otu00155	day 2	0.000354	Firmicutes	Clostridia	Peptostreptococcales- Tissierellales	Peptostreptococcales- Tissierellales_fa	Anaerococcus
Otu00030	day 2	0.002128	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Anaerosporebacter
Otu01250	day 2	0.000753	Firmicutes	Negativicutes	Veillonellales- Selenomonadales	Selenomonadaceae	Anaerovibrio
Otu02421	day 2	0.000716	Firmicutes	Bacilli	Bacillales	Bacillaceae	Anoxybacillus
Otu00172	day 2	0.000103	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Atopostipes
Otu00042	day 2	1.178414	Firmicutes	Bacilli	Bacillales	Bacillales_unclassified	Bacillales_unclassified
Otu01665	day 2	0.002708	Firmicutes	Bacilli	Bacilli_unclassified	Bacilli_unclassified	Bacilli_unclassified
Otu00069	day 2	0.00021	Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidales_unclassified	Bacteroidales_unclassified
Otu02014	day 2	0.00021	Actinobacteriota	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacteriaceae_unclassified
Otu00034	day 2	0.017246	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Blautia
Otu01084	day 2	0.000113	Bacteroidota	Bacteroidia	Bacteroidales	Muribaculaceae	CAG-873
Otu00411	day 2	0.000116	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Candidatus_Soleaferrea
Otu00092	day 2	0.000351	Verrucomicrobia	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	Candidatus_Udaeobacter
Otu01593	day 2	0.000341	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Carnobacterium
Otu00550	day 2	0.000113	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Caulobacteraceae_unclassified
Otu01386	day 2	0.00012	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Cellulosilyticum

Table 3: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu00337	day 2	0.000564	Cyanobacteria	Cyanobacteriia	Chloroplast	Chloroplast_fa	Chloroplast_ge
Otu00415	day 2	0.000116	Bacteroidota	Bacteroidia	Flavobacteriales	Weeksellaceae	Chryseobacterium
Otu01903	day 2	0.000534	Firmicutes	Clostridia	Clostridia_unclassified	Clostridia_unclassified	Clostridia_unclassified
Otu02991	day 2	0.000957	Firmicutes	Clostridia	Clostridia_vadinBB60_group	Clostridia_vadinBB60_group_fa	Clostridia_vadinBB60_group_ge
Otu00035	day 2	0.049401	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridiaceae_unclassified
Otu00004	day 2	10.17851	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Clostridioides
Otu00005	day 2	7.161936	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium_sensu_stricto_1
Otu00942	day 2	0.000301	Actinobacteriota	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Collinsella
Otu00232	day 2	0.000337	Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamonadaceae	Comamonadaceae_unclassified
Otu00344	day 2	0.000668	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Coprococcus
Otu00378	day 2	0.001377	Actinobacteriota	Actinobacteria	Corynebacteriales	Corynebacteriaceae	Corynebacterium
Otu00028	day 2	0.00012	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Eisenbergiella
Otu01007	day 2	0.000231	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Enhydrobacter
Otu00134	day 2	0.014944	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriales_unclassified	Enterobacteriales_unclassified
Otu00002	day 2	56.01674	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacteriaceae_unclassified
Otu01304	day 2	0.000345	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcaceae_unclassified
Otu00009	day 2	0.030848	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus
Otu00175	day 2	0.002154	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Faecalibacterium
Otu00565	day 2	0.000226	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Anaerovoracaceae	Family_XIII_AD3011_group
Otu00107	day 2	0.000105	Firmicutes	Clostridia	Clostridia_or	Hungateiclostridiaceae	Fastidiosipila
Otu00085	day 2	0.00012	Bacteroidota	Bacteroidia	Bacteroidales	Dysgonomonadaceae	Fermentimonas
Otu00113	day 2	0.00094	Firmicutes	Firmicutes_unclassified	Firmicutes_unclassified	Firmicutes_unclassified	Firmicutes_unclassified
Otu00008	day 2	0.000361	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	Flavonifractor

Table 3: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu02679	day 2	0.000956	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Fructobacillus
Otu01352	day 2	0.000113	Actinobacteriota	Thermoleophila	Gaiellales	Gaiellaceae	Gaiella
Otu00503	day 2	0.000618	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria _unclassified	Gammaproteobacteria_ unclassified	Gammaproteobacteria_ unclassified
Otu02649	day 2	0.000118	Firmicutes	Bacilli	Bacillales	Bacillaceae	Geobacillus
Otu00866	day 2	0.000382	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Hafniaceae	Hafnia- Obesumbacterium
Otu02513	day 2	0.002393	Firmicutes	Clostridia	Peptostreptococcales- Tissierellales	Peptostreptococcales- Tissierellales fa	Helcococcus
Otu00015	day 2	0.001724	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Histophilus
Otu02399	day 2	0.000462	Firmicutes	Bacilli	Erysipelotrichales	Erysipelotrichaceae	Holdemanella
Otu00286	day 2	0.000103	Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Ignavigranum
Otu00445	day 2	0.001556	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Incertae_Sedis
Otu00089	day 2	0.00021	Firmicutes	Clostridia	Peptostreptococcales- Tissierellales	Peptostreptococcaceae	Intestinibacter
Otu00018	day 2	0.000446	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	Intestinimonas
Otu00065	day 2	0.00044	Firmicutes	Bacilli	Izomoplasmatales	Izomoplasmatales_fa	Izomoplasmatales_ge
Otu01599	day 2	0.00116	Chloroflexi	KD4-96	KD4-96_or	KD4-96_fa	KD4-96_ge
Otu02183	day 2	0.001464	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Klebsiella
Otu00084	day 2	0.000113	Firmicutes	Bacilli	Bacillales	Planococcaceae	Kurthia
Otu01187	day 2	0.002393	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae_ FCS020_group
Otu00162	day 2	0.000462	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae_ NK4A136_group
Otu01237	day 2	0.000347	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae_ NK4B4_group
Otu00905	day 2	0.00012	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae_ge
Otu00011	day 2	0.07896	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae_ unclassified
Otu00818	day 2	0.001281	Firmicutes	Bacilli	Lactobacillales	Lactobacillales_ unclassified	Lactobacillales_ unclassified

Table 3: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu00716	day 2	0.000103	Actinobacteriota	Actinobacteria	Corynebacteriales	Corynebacteriaceae	Lawsonella
Otu00847	day 2	0.000273	Bacteroidota	Bacteroidia	Sphingobacteriales	Lentimicrobiaceae	Lentimicrobiaceae_ge
Otu00128	day 2	0.00351	Fusobacteriota	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichiaceae_unclassified
Otu01775	day 2	0.000906	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Leuconostoc
Otu02915	day 2	0.001355	Firmicutes	Limnochordia	Limnochordia_unclassified	Limnochordia_unclassified	Limnochordia_unclassified
Otu00399	day 2	0.000116	Firmicutes	Clostridia	Clostridia_or	Hungateiclostridiaceae	Mageibacillus
Otu00809	day 2	0.000137	Proteobacteria	Gammaproteobacteria	Burkholderiales	Oxalobacteraceae	Massilia
Otu00048	day 2	0.000255	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Megasphaera
Otu00056	day 2	0.001959	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobrevibacter
Otu00434	day 2	0.000137	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanosphaera
Otu02123	day 2	0.000567	Actinobacteriota	Actinobacteria	Micrococcales	Microbacteriaceae	Microbacteriaceae_unclassified
Otu00826	day 2	0.001675	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Anaerovoracaceae	Mogibacterium
Otu00062	day 2	0.000151	Bacteroidota	Bacteroidia	Bacteroidales	Muribaculaceae	Muribaculaceae_ge
Otu01330	day 2	0.000236	Actinobacteriota	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium
Otu00741	day 2	0.001205	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	NK4A214_group
Otu00448	day 2	0.000513	Proteobacteria	Gammaproteobacteria	Burkholderiales	Neisseriaceae	Neisseria
Otu02779	day 2	0.000151	Nitrospirota	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrospira
Otu00326	day 2	0.000137	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium
Otu00659	day 2	0.000116	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Ochrobactrum
Otu02448	day 2	0.001959	Proteobacteria	Gammaproteobacteria	Burkholderiales	Alcaligenaceae	Oligella
Otu02395	day 2	0.000118	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	Oscillospiraceae_unclassified
Otu00019	day 2	0.000103	Firmicutes	Clostridia	Oscillospirales	Oscillospirales_fa	Oscillospirales_ge
Otu01478	day 2	0.000116	Firmicutes	Clostridia	Oscillospirales	Oscillospirales_unclassified	Oscillospirales_unclassified
Otu00044	day 2	0.000151	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Paludicola

Table 3: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu00006	day 2	0.002405	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Pasteurellaceae_unclassified
Otu00339	day 2	0.000116	Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamonadaceae	Paucibacter
Otu00027	day 2	0.00034	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Pediococcus
Otu00157	day 2	0.003924	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Peptostreptococcaceae_unclassified
Otu00202	day 2	0.002062	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcales-Tissierellales_fa	Peptostreptococcales-Tissierellales_fa_unclassified
Otu00050	day 2	0.997479	Firmicutes	Bacilli	Bacillales	Planococcaceae	Planococcaceae_unclassified
Otu02585	day 2	0.006048	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
Otu00108	day 2	0.003282	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_NK3B31_group
Otu00147	day 2	0.00021	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_UCG-003
Otu02509	day 2	0.002393	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Proteiniclasticum
Otu00079	day 2	0.002074	Bacteroidota	Bacteroidia	Bacteroidales	Dysgonomonadaceae	Proteiniphilum
Otu00131	day 2	0.003112	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
Otu00362	day 2	0.001108	Firmicutes	Bacilli	RF39	RF39_fa	RF39_ge
Otu01739	day 2	0.000118	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Rhizobiaceae_unclassified
Otu00098	day 2	0.000223	Bacteroidota	Bacteroidia	Bacteroidales	Rikenellaceae	Rikenellaceae_RC9_gut_group
Otu00656	day 2	0.000116	Methylomirabilota	Methylomirabilia	Rokubacteriales	Rokubacteriales_fa	Rokubacteriales_ge
Otu00053	day 2	0.001779	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Romboutsia
Otu00577	day 2	0.000103	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Ruminococcus
Otu00126	day 2	0.000342	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Salinicola
Otu00355	day 2	0.000376	Firmicutes	Bacilli	Erysipelotrichales	Erysipelatoclostridiaceae	Sharpea
Otu00059	day 2	0.000151	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas
Otu00995	day 2	0.000118	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingorhabdus
Otu00455	day 2	0.000113	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	Staphylococcaceae_unclassified

Table 3: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu00020	day 2	0.01986	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	Staphylococcus
Otu00099	day 2	0.13048	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
Otu00129	day 2	0.00113	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Succinivibrio
Otu02319	day 2	0.000904	Proteobacteria	Gammaproteobacteria	Burkholderiales	Sutterellaceae	Sutterella
Otu00022	day 2	0.005004	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Terrisporobacter
Otu01753	day 2	0.002051	Spirochaetota	Spirochaetia	Spirochaetales	Spirochaetaceae	Treponema
Otu00525	day 2	0.000118	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Trichococcus
Otu00340	day 2	0.000218	Actinobacteriota	Actinobacteria	Actinomycetales	Actinomycetaceae	Trueperella
Otu00013	day 2	0.021267	Firmicutes	Bacilli	Erysipelotrichales	Erysipelotrichaceae	Turicibacter
Otu00043	day 2	0.002566	Proteobacteria	Gammaproteobacteria	Burkholderiales	Methylophilaceae	UBA6140
Otu00266	day 2	0.002274	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	UCG-002
Otu00409	day 2	0.000103	Firmicutes	Bacilli	Erysipelotrichales	Erysipelatoclostridiaceae	UCG-004
Otu00158	day 2	0.001278	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	UCG-005
Otu00137	day 2	0.000347	Firmicutes	Clostridia	Oscillospirales	Butyricicoccaceae	UCG-008
Otu00966	day 2	0.001058	Firmicutes	Clostridia	Oscillospirales	UCG-010	UCG-010_ge
Otu00181	day 2	0.000137	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria Incertae Sedis	Unknown_Family	Unknown_Family_ge
Otu00007	day 2	0.006593	Firmicutes	Bacilli	Mycoplasmatales	Mycoplasmataceae	Ureaplasma
Otu00783	day 2	0.001078	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio
Otu00179	day 2	0.000118	Verrucomicrobiota	Kiritimatiellae	WCHB1-41	WCHB1-41_fa	WCHB1-41_ge
Otu00058	day 2	0.001473	Proteobacteria	Gammaproteobacteria	Cardiobacteriales	Wohlfahrtiimonadaceae	Wohlfahrtiimonas
Otu00527	day 2	0.000546	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Xanthomonadaceae_unclassified
Otu00309	day 2	0.00012	Actinobacteriota	Actinobacteria	Micrococcales	Micrococcaceae	Yaniella
Otu01228	day 2	0.000603	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Yersiniaceae	Yersinia
Otu00667	day 2	0.000105	Verrucomicrobiota	Lentisphaeria	Oligosphaerales	Oligosphaeraceae	Z20

Table 3: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu02347	day 2	0.001554	Planctomycetota	Planctomycetes	Pirellulales	Pirellulaceae	p-1088-a5_gut_group
Otu00024	day 2	0.000116	Bacteroidota	Bacteroidia	Bacteroidales	p-251-o5	p-251-o5_ge
Otu02346	day 2	0.003688	Firmicutes	Bacilli	Bacillales	Planococcaceae	uncultured
Otu02571	day 2	0.002154	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	uncultured
Otu00095	day 2	0.001734	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Selenomonadaceae	uncultured
Otu00174	day 2	0.0011	Synergistota	Synergistia	Synergistales	Synergistaceae	uncultured
Otu00664	day 2	0.000118	Gemmatimonadota	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	uncultured
Otu00380	day 2	0.000105	Firmicutes	Limnochordia	Limnochordales	uncultured	uncultured_ge
Otu02660	day 4	0.000102	Bdellovibrionota	Oligoflexia	0319-6G20	0319-6G20_fa	0319-6G20_ge
Otu00950	day 4	0.000145	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Acetitomaculum
Otu00111	day 4	0.000272	Firmicutes	Bacilli	Acholeplasmatales	Acholeplasmataceae	Acholeplasma
Otu00495	day 4	9.35E-05	Actinobacteriota	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces
Otu00307	day 4	0.0001	Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Aerococcus
Otu00030	day 4	0.013717	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Anaerosporebacter
Otu00163	day 4	0.004987	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Anaerostipes
Otu00042	day 4	0.014911	Firmicutes	Bacilli	Bacillales	Bacillales_unclassified	Bacillales_unclassified
Otu01665	day 4	0.000795	Firmicutes	Bacilli	Bacilli_unclassified	Bacilli_unclassified	Bacilli_unclassified
Otu00069	day 4	0.0001	Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidales_unclassified	Bacteroidales_unclassified
Otu00034	day 4	0.07677	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Blautia
Otu01155	day 4	0.000102	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	CAG-56
Otu00411	day 4	0.004687	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Candidatus_Soleaferrea
Otu00092	day 4	0.000281	Verrucomicrobiota	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	Candidatus_Udaeobacter
Otu01593	day 4	0.000344	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Carnobacterium
Otu02956	day 4	0.000115	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	Christensenellaceae_R-7_group



Table 3: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu01903	day 4	0.000115	Firmicutes	Clostridia	Clostridia_unclassified	Clostridia_unclassified	Clostridia_unclassified
Otu00035	day 4	0.148607	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridiaceae_unclassified
Otu00004	day 4	0.579146	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Clostridioides
Otu00005	day 4	0.511934	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium_sensu_stricto_1
Otu01776	day 4	0.000279	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium_sensu_stricto_13
Otu00814	day 4	8.87E-05	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	Colidextribacter
Otu00232	day 4	8.48E-05	Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamonadaceae	Comamonadaceae_unclassified
Otu00378	day 4	0.0002	Actinobacteriota	Actinobacteria	Corynebacteriales	Corynebacteriaceae	Corynebacterium
Otu00549	day 4	0.000145	Desulfobacterota	Desulfovibrionia	Desulfovibrionales	Desulfovibrionaceae	Desulfovibrio
Otu00028	day 4	0.000373	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Eisenbergiella
Otu00431	day 4	0.000272	Bacteroidota	Bacteroidia	Flavobacteriales	Weeksellaceae	Empedobacter
Otu00134	day 4	0.124352	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacterales_unclassified	Enterobacterales_unclassified
Otu00002	day 4	22.23073	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Enterobacteriaceae_unclassified
Otu01304	day 4	0.001368	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcaceae_unclassified
Otu00009	day 4	0.092488	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus
Otu00033	day 4	0.037966	Firmicutes	Bacilli	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_ge
Otu00057	day 4	0.000102	Bacteroidota	Bacteroidia	Bacteroidales	F082	F082_ge
Otu00332	day 4	8.87E-05	Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Facklamia
Otu00107	day 4	0.000803	Firmicutes	Clostridia	Clostridia_or	Hungateiclostridiaceae	Fastidiosipila
Otu00085	day 4	8.87E-05	Bacteroidota	Bacteroidia	Bacteroidales	Dysgonomonadaceae	Fermentimonas
Otu00113	day 4	0.000725	Firmicutes	Firmicutes_unclassified	Firmicutes_unclassified	Firmicutes_unclassified	Firmicutes_unclassified
Otu00008	day 4	1.974946	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	Flavonifractor
Otu02679	day 4	0.00079	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Fructobacillus

Table 3: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu00324	day 4	0.000234	Fusobacteriota	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium
Otu01067	day 4	8.87E-05	Actinobacteriota	Thermoleophilia	Gaiellales	Gaiellales_unclassified	Gaiellales_unclassified
Otu02649	day 4	0.000115	Firmicutes	Bacilli	Bacillales	Bacillaceae	Geobacillus
Otu00072	day 4	0.017586	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Harryflintia
Otu00015	day 4	0.000682	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Histophilus
Otu00445	day 4	0.016673	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Incertae_Sedis
Otu00089	day 4	9.35E-05	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Intestinibacter
Otu00018	day 4	0.108491	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	Intestinimonas
Otu00065	day 4	0.000541	Firmicutes	Bacilli	Izemoplasmatales	Izemoplasmatales_fa	Izemoplasmatales_ge
Otu00216	day 4	0.000115	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	Jeotgalicoccus
Otu02183	day 4	0.000565	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Klebsiella
Otu00084	day 4	0.000182	Firmicutes	Bacilli	Bacillales	Planococcaceae	Kurthia
Otu01280	day 4	8.87E-05	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae_NK3A20_group
Otu00011	day 4	0.913511	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae_unclassified
Otu00818	day 4	0.000768	Firmicutes	Bacilli	Lactobacillales	Lactobacillales_unclassified	Lactobacillales_unclassified
Otu00128	day 4	0.000799	Fusobacteriota	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichiaceae_unclassified
Otu01775	day 4	0.004054	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Leuconostoc
Otu00056	day 4	0.0001	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobrevibacter
Otu01252	day 4	0.0001	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanothermobacteriaceae	Methanothermobacter
Otu01031	day 4	0.000229	Actinobacteriota	Actinobacteria	Micrococcales	Micrococcaceae	Micrococcus
Otu01837	day 4	9.35E-05	Firmicutes	Clostridia	Monoglobales	Monoglobaceae	Monoglobus
Otu00062	day 4	0.000292	Bacteroidota	Bacteroidia	Bacteroidales	Muribaculaceae	Muribaculaceae_ge
Otu00346	day 4	0.000102	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Oblitimonas
Otu00019	day 4	0.024915	Firmicutes	Clostridia	Oscillospirales	Oscillospirales_fa	Oscillospirales_ge

Table 3: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu01478	day 4	0.000279	Firmicutes	Clostridia	Oscillospirales	Oscillospirales_unclassified	Oscillospirales_unclassified
Otu00044	day 4	0.000281	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Paludicola
Otu00006	day 4	0.00464	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Pasteurellaceae_unclassified
Otu00027	day 4	0.00094	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Pediococcus
Otu00157	day 4	0.001621	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Peptostreptococcaceae_unclassified
Otu00202	day 4	0.000267	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcales-Tissierellales_fa	Peptostreptococcales-Tissierellales_fa_unclassified
Otu00173	day 4	8.87E-05	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_UCG-001
Otu00079	day 4	9.35E-05	Bacteroidota	Bacteroidia	Bacteroidales	Dysgonomonadaceae	Proteiniphilum
Otu00206	day 4	0.000102	Actinobacteriota	Actinobacteria	Micrococcales	Micrococcaceae	Pseudarthrobacter
Otu00131	day 4	0.00764	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
Otu00362	day 4	0.000749	Firmicutes	Bacilli	RF39	RF39_fa	RF39_ge
Otu02989	day 4	0.000115	Actinobacteriota	Actinobacteria	Corynebacteriales	Nocardiaceae	Rhodococcus
Otu00098	day 4	0.000367	Bacteroidota	Bacteroidia	Bacteroidales	Rikenellaceae	Rikenellaceae_RC9_gut_group
Otu00053	day 4	0.000234	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Romboutsia
Otu00090	day 4	0.020511	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Ruminococcaceae_unclassified
Otu01711	day 4	0.000115	Actinobacteriota	Actinobacteria	Micrococcales	Sanguibacteraceae	Sanguibacter
Otu00355	day 4	0.000115	Firmicutes	Bacilli	Erysipelotrichales	Erysipelatoclostridiaceae	Sharpea
Otu01430	day 4	9.35E-05	Actinobacteriota	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	Solirubrobacter
Otu00059	day 4	0.000102	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas
Otu00020	day 4	0.057831	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	Staphylococcus
Otu00099	day 4	0.063835	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
Otu00849	day 4	8.87E-05	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Subdoligranulum

Table 3: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu00129	day 4	0.000203	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Succinivibrio
Otu00022	day 4	0.073649	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Terrisporobacter
Otu01368	day 4	0.000189	Deinococcota	Deinococci	Thermales	Thermaceae	Thermus
Otu01753	day 4	0.000453	Spirochaetota	Spirochaetia	Spirochaetales	Spirochaetaceae	Treponema
Otu00340	day 4	9.35E-05	Actinobacteriota	Actinobacteria	Actinomycetales	Actinomycetaceae	Trueperella
Otu00013	day 4	0.99161	Firmicutes	Bacilli	Erysipelotrichales	Erysipelotrichaceae	Turicibacter
Otu00043	day 4	9.35E-05	Proteobacteria	Gammaproteobacteria	Burkholderiales	Methylophilaceae	UBA6140
Otu00158	day 4	0.000519	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	UCG-005
Otu00137	day 4	0.000182	Firmicutes	Clostridia	Oscillospirales	Butyricicoccaceae	UCG-008
Otu00966	day 4	0.000247	Firmicutes	Clostridia	Oscillospirales	UCG-010	UCG-010_ge
Otu00007	day 4	0.001028	Firmicutes	Bacilli	Mycoplasmatales	Mycoplasmataceae	Ureaplasma
Otu00179	day 4	8.48E-05	Verrucomicrobiota	Kiritimatiellae	WCHB1-41	WCHB1-41_fa	WCHB1-41_ge
Otu00058	day 4	8.87E-05	Proteobacteria	Gammaproteobacteria	Cardiobacteriales	Wohlfahrtiimonadaceae	Wohlfahrtiimonas
Otu00309	day 4	0.000102	Actinobacteriota	Actinobacteria	Micrococcales	Micrococcaceae	Yaniella
Otu00063	day 4	9.35E-05	Bacteroidota	Bacteroidia	Bacteroidales	Rikenellaceae	dgA-11_gut_group
Otu00174	day 4	0.000189	Synergistota	Synergistia	Synergistales	Synergistaceae	uncultured
Otu00472	day 4	9.35E-05	Actinobacteriota	Thermoleophilia	Gaiellales	uncultured	uncultured_ge
Otu00052	day 7-8	0.000295	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter
Otu00300	day 7-8	0.000182	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Actinobacillus
Otu00683	day 7-8	8.98E-05	Proteobacteria	Gammaproteobacteria	Burkholderiales	Alcaligenaceae	Alcaligenes
Otu00030	day 7-8	0.005826	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Anaerosporebacter
Otu00163	day 7-8	0.002399	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Anaerostipes
Otu01665	day 7-8	0.031827	Firmicutes	Bacilli	Bacilli_unclassified	Bacilli_unclassified	Bacilli_unclassified
Otu02318	day 7-8	0.001176	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified
Otu00069	day 7-8	0.000552	Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidales_unclassified	Bacteroidales_unclassified

Table 3: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu00386	day 7-8	0.000113	Bacteroidota	Bacteroidia	Bacteroidia_unclassified	Bacteroidia_unclassified	Bacteroidia_unclassified
Otu00034	day 7-8	0.106664	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Blautia
Otu01155	day 7-8	0.002714	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	CAG-56
Otu00127	day 7-8	0.000213	Campilobacterota	Campylobacteria	Campylobacterales	Campylobacteraceae	Campylobacter
Otu00411	day 7-8	0.001424	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Candidatus_Soleaferrea
Otu00337	day 7-8	0.002033	Cyanobacteria	Cyanobacteriia	Chloroplast	Chloroplast_fa	Chloroplast_ge
Otu02956	day 7-8	9.38E-05	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	Christensenellaceae_R-7_group
Otu00415	day 7-8	0.000113	Bacteroidota	Bacteroidia	Flavobacteriales	Weeksellaceae	Chryseobacterium
Otu02760	day 7-8	9.39E-05	Firmicutes	Clostridia	Clostridia_UCG-014	Clostridia_UCG-014_fa	Clostridia_UCG-014_ge
Otu01903	day 7-8	0.003744	Firmicutes	Clostridia	Clostridia_unclassified	Clostridia_unclassified	Clostridia_unclassified
Otu02991	day 7-8	9.39E-05	Firmicutes	Clostridia	Clostridia_vadinBB60_group	Clostridia_vadinBB60_group_fa	Clostridia_vadinBB60_group_ge
Otu00035	day 7-8	0.113813	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridiaceae_unclassified
Otu00004	day 7-8	0.631157	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Clostridioides
Otu00005	day 7-8	0.100666	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium_sensu_stricto_1
Otu01776	day 7-8	0.004079	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium_sensu_stricto_13
Otu00344	day 7-8	9.23E-05	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Coprococcus
Otu00378	day 7-8	0.000184	Actinobacteriota	Actinobacteria	Corynebacteriales	Corynebacteriaceae	Corynebacterium
Otu00028	day 7-8	0.000272	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Eisenbergiella
Otu00134	day 7-8	0.03363	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriales_unclassified	Enterobacteriales_unclassified
Otu00002	day 7-8	16.05971	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Enterobacteriaceae_unclassified
Otu01304	day 7-8	0.02289	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcaceae_unclassified
Otu00009	day 7-8	2.789166	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus
Otu00033	day 7-8	0.450431	Firmicutes	Bacilli	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_ge

Table 3: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu00057	day 7-8	0.000158	Bacteroidota	Bacteroidia	Bacteroidales	F082	F082_ge
Otu00332	day 7-8	9.23E-05	Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Facklamia
Otu00085	day 7-8	0.000565	Bacteroidota	Bacteroidia	Bacteroidales	Dysgonomonadaceae	Fermentimonas
Otu00113	day 7-8	0.000436	Firmicutes	Firmicutes_unclassified	Firmicutes_unclassified	Firmicutes_unclassified	Firmicutes_unclassified
Otu00008	day 7-8	0.900159	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	Flavonifractor
Otu02679	day 7-8	8.98E-05	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Fructobacillus
Otu00072	day 7-8	0.103472	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Harryflintia
Otu00015	day 7-8	0.000271	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Histophilus
Otu00445	day 7-8	0.016843	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Incertae_Sedis
Otu00089	day 7-8	9.38E-05	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Intestinibacter
Otu00018	day 7-8	0.506216	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	Intestinimonas
Otu00065	day 7-8	0.001051	Firmicutes	Bacilli	Izemoplasmatales	Izemoplasmatales_fa	Izemoplasmatales_ge
Otu00216	day 7-8	9.38E-05	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	Jeotgalicoccus
Otu02183	day 7-8	0.000589	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Klebsiella
Otu00084	day 7-8	9.04E-05	Firmicutes	Bacilli	Bacillales	Planococcaceae	Kurthia
Otu00162	day 7-8	0.000184	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae_NK4A136_group
Otu00011	day 7-8	1.283176	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae_unclassified
Otu00818	day 7-8	0.036936	Firmicutes	Bacilli	Lactobacillales	Lactobacillales_unclassified	Lactobacillales_unclassified
Otu00128	day 7-8	9.23E-05	Fusobacteriota	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichiaceae_unclassified
Otu01775	day 7-8	0.002621	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Leuconostoc
Otu02915	day 7-8	9.04E-05	Firmicutes	Limnochordia	Limnochordia_unclassified	Limnochordia_unclassified	Limnochordia_unclassified
Otu00048	day 7-8	0.000387	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Megasphaera
Otu00434	day 7-8	9.38E-05	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanosphaera
Otu00062	day 7-8	0.000271	Bacteroidota	Bacteroidia	Bacteroidales	Muribaculaceae	Muribaculaceae_ge

Table 3: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu00765	day 7-8	9.04E-05	Proteobacteria	Gammaproteobacteria	Burkholderiales	Nitrosomonadaceae	Nitrosomonas
Otu01440	day 7-8	0.000226	Bacteroidota	Bacteroidia	Bacteroidales	Marinifilaceae	Odoribacter
Otu00839	day 7-8	9.04E-05	Actinobacteriota	Coriobacteriia	Coriobacteriales	Atopobiaceae	Olsenella
Otu00310	day 7-8	0.000107	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	Oscillibacter
Otu02395	day 7-8	0.00099	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	Oscillospiraceae_unclassified
Otu00019	day 7-8	0.469385	Firmicutes	Clostridia	Oscillospirales	Oscillospirales_fa	Oscillospirales_ge
Otu01478	day 7-8	0.007852	Firmicutes	Clostridia	Oscillospirales	Oscillospirales_unclassified	Oscillospirales_unclassified
Otu00044	day 7-8	0.004173	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Paludicola
Otu02021	day 7-8	9.38E-05	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Paracoccus
Otu00260	day 7-8	0.000158	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Pasteurella
Otu00006	day 7-8	0.000381	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Pasteurellaceae_unclassified
Otu00027	day 7-8	0.02066	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Pediococcus
Otu00157	day 7-8	0.001266	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Peptostreptococcaceae_unclassified
Otu00251	day 7-8	0.000113	Firmicutes	Negativicutes	Acidaminococcales	Acidaminococcaceae	Phascolarctobacterium
Otu00050	day 7-8	9.39E-05	Firmicutes	Bacilli	Bacillales	Planococcaceae	Planococcaceae_unclassified
Otu02585	day 7-8	0.000297	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
Otu00108	day 7-8	0.000384	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_NK3B31_group
Otu00147	day 7-8	9.38E-05	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_UCG-003
Otu00745	day 7-8	0.000185	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_unclassified
Otu00131	day 7-8	0.00053	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
Otu01273	day 7-8	9.04E-05	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Psychrobacter
Otu00362	day 7-8	0.000458	Firmicutes	Bacilli	RF39	RF39_fa	RF39_ge
Otu00098	day 7-8	0.000186	Bacteroidota	Bacteroidia	Bacteroidales	Rikenellaceae	Rikenellaceae_RC9_gut_group

Table 3: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu00053	day 7-8	0.000275	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Romboutsia
Otu01583	day 7-8	9.04E-05	Actinobacteriota	Actinobacteria	Micrococcales	Micrococcaceae	Rothia
Otu00090	day 7-8	0.031647	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Ruminococcaceae_unclassified
Otu00126	day 7-8	0.000205	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Salinicola
Otu00020	day 7-8	0.00602	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	Staphylococcus
Otu00177	day 7-8	0.000709	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas
Otu00099	day 7-8	0.018085	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
Otu00129	day 7-8	0.000248	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Succinivibrio
Otu00022	day 7-8	0.059751	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Terrisporobacter
Otu01753	day 7-8	0.00097	Spirochaetota	Spirochaetia	Spirochaetales	Spirochaetaceae	Treponema
Otu00340	day 7-8	0.000374	Actinobacteriota	Actinobacteria	Actinomycetales	Actinomycetaceae	Trueperella
Otu00013	day 7-8	2.248268	Firmicutes	Bacilli	Erysipelotrichales	Erysipelotrichaceae	Turicibacter
Otu00043	day 7-8	0.000301	Proteobacteria	Gammaproteobacteria	Burkholderiales	Methylophilaceae	UBA6140
Otu00266	day 7-8	0.000251	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	UCG-002
Otu00409	day 7-8	0.000158	Firmicutes	Bacilli	Erysipelotrichales	Erysipelatoclostridiaceae	UCG-004
Otu00158	day 7-8	0.000565	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	UCG-005
Otu00137	day 7-8	0.000186	Firmicutes	Clostridia	Oscillospirales	Butyricicoccaceae	UCG-008
Otu00181	day 7-8	9.38E-05	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria Incertae Sedis	Unknown_Family	Unknown_Family_ge
Otu00007	day 7-8	0.000466	Firmicutes	Bacilli	Mycoplasmatales	Mycoplasmataceae	Ureaplasma
Otu00402	day 7-8	0.000158	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrionaceae_unclassified
Otu00179	day 7-8	0.000185	Verrucomicrobiota	Kiritimatiellae	WCHB1-41	WCHB1-41_fa	WCHB1-41_ge
Otu00095	day 7-8	0.000113	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Selenomonadaceae	uncultured
Otu01218	day 7-8	0.000107	Firmicutes	Clostridia	Peptococcales	Peptococcaceae	uncultured



Table 3: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu00111	day 14-15	0.000127	Firmicutes	Bacilli	Acholeplasmatales	Acholeplasmataceae	Acholeplasma
Otu03009	day 14-15	0.000103	Firmicutes	Bacilli	Acholeplasmatales	Acholeplasmataceae	Acholeplasmataceae_unclassified
Otu00444	day 14-15	0.001835	Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriales_unclassified	Acidobacteriales_unclassified
Otu00052	day 14-15	0.002457	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter
Otu00300	day 14-15	0.002486	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Actinobacillus
Otu00155	day 14-15	0.002622	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcales-Tissierellales fa	Anaerococcus
Otu00030	day 14-15	0.792336	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Anaerosporobacter
Otu00163	day 14-15	0.181495	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Anaerostipes
Otu01250	day 14-15	0.000393	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Selenomonadaceae	Anaerovibrio
Otu00042	day 14-15	0.000131	Firmicutes	Bacilli	Bacillales	Bacillales_unclassified	Bacillales_unclassified
Otu01665	day 14-15	0.002034	Firmicutes	Bacilli	Bacilli_unclassified	Bacilli_unclassified	Bacilli_unclassified
Otu02318	day 14-15	0.000507	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified
Otu00069	day 14-15	0.002575	Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidales_unclassified	Bacteroidales_unclassified
Otu03137	day 14-15	0.001311	Bdellovibrionota	Bdellovibrionia	Bdellovibrionales	Bdellovibrionaceae	Bdellovibrio
Otu00034	day 14-15	0.881832	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Blautia
Otu01155	day 14-15	0.024253	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	CAG-56
Otu00127	day 14-15	0.00236	Campilobacterota	Campylobacteria	Campylobacterales	Campylobacteraceae	Campylobacter
Otu00411	day 14-15	0.024508	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Candidatus_Soleaferrea
Otu01387	day 14-15	0.000127	Acidobacteriota	Acidobacteriae	Solibacterales	Solibacteraceae	Candidatus_Solibacter
Otu00092	day 14-15	0.000114	Verrucomicrobiota	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	Candidatus_Udaeobacter

Table 3: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu00337	day 14-15	0.033246	Cyanobacteria	Cyanobacteriia	Chloroplast	Chloroplast_fa	Chloroplast_ge
Otu02956	day 14-15	0.00102	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	Christensenellaceae_R-7_group
Otu02760	day 14-15	0.002041	Firmicutes	Clostridia	Clostridia_UCG-014	Clostridia_UCG-014_fa	Clostridia_UCG-014_ge
Otu01903	day 14-15	0.00697	Firmicutes	Clostridia	Clostridia_unclassified	Clostridia_unclassified	Clostridia_unclassified
Otu02991	day 14-15	0.000114	Firmicutes	Clostridia	Clostridia_vadinBB60_group	Clostridia_vadinBB60_group_fa	Clostridia_vadinBB60_group_ge
Otu00035	day 14-15	0.696437	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridiaceae_unclassified
Otu00004	day 14-15	0.756092	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Clostridioides
Otu00005	day 14-15	1.065823	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium_sensu_stricto_1
Otu01776	day 14-15	0.005062	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium_sensu_stricto_13
Otu00344	day 14-15	0.00012	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Coprococcus
Otu00226	day 14-15	0.001835	Actinobacteriota	Actinobacteria	Corynebacteriales	Corynebacteriaceae	Corynebacteriaceae_unclassified
Otu00378	day 14-15	0.00011	Actinobacteriota	Actinobacteria	Corynebacteriales	Corynebacteriaceae	Corynebacterium
Otu00028	day 14-15	0.042415	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Eisenbergiella
Otu02923	day 14-15	0.000131	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Enterobacter
Otu00134	day 14-15	0.065746	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacterales_unclassified	Enterobacterales_unclassified
Otu00002	day 14-15	12.38528	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Enterobacteriaceae_unclassified
Otu01304	day 14-15	0.001573	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcaceae_unclassified
Otu00009	day 14-15	0.575101	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus
Otu00271	day 14-15	0.000918	Firmicutes	Bacilli	Erysipelotrichales	Erysipelatoclostridiaceae	Erysipelotrichaceae_UCG-002
Otu00033	day 14-15	0.75815	Firmicutes	Bacilli	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_ge

Table 3: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu00332	day 14-15	0.000131	Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Facklamia
Otu00085	day 14-15	0.00067	Bacteroidota	Bacteroidia	Bacteroidales	Dysgonomonadaceae	Fermentimonas
Otu00113	day 14-15	0.000105	Firmicutes	Firmicutes_unclassified	Firmicutes_unclassified	Firmicutes_unclassified	Firmicutes_unclassified
Otu00008	day 14-15	0.907	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	Flavonifractor
Otu00674	day 14-15	0.000131	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Frisingicoccus
Otu02679	day 14-15	0.000127	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Fructobacillus
Otu00324	day 14-15	0.00118	Fusobacteriota	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium
Otu00072	day 14-15	0.192436	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Harryflintia
Otu00015	day 14-15	0.000105	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Histophilus
Otu02399	day 14-15	0.000655	Firmicutes	Bacilli	Erysipelotrichales	Erysipelotrichaceae	Holdemanella
Otu00445	day 14-15	0.01554	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Incertae_Sedis
Otu00089	day 14-15	0.0056	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Intestinibacter
Otu00018	day 14-15	0.6907	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	Intestinimonas
Otu00065	day 14-15	0.000784	Firmicutes	Bacilli	Izemoplasmatales	Izemoplasmatales_fa	Izemoplasmatales_ge
Otu02183	day 14-15	0.000457	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Klebsiella
Otu00162	day 14-15	0.000262	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae_NK4A136_group
Otu00011	day 14-15	3.026643	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae_unclassified
Otu00818	day 14-15	0.006312	Firmicutes	Bacilli	Lactobacillales	Lactobacillales_unclassified	Lactobacillales_unclassified
Otu01775	day 14-15	0.000508	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Leuconostoc
Otu00623	day 14-15	0.000103	Firmicutes	Bacilli	Lactobacillales	Listeriaceae	Listeria

Table 3: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu00048	day 14-15	0.000918	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Megasphaera
Otu00056	day 14-15	0.000524	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobrevibacter
Otu00434	day 14-15	0.000114	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanosphaera
Otu00826	day 14-15	0.00105	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Anaerovoracaceae	Mogibacterium
Otu00062	day 14-15	0.000105	Bacteroidota	Bacteroidia	Bacteroidales	Muribaculaceae	Muribaculaceae_ge
Otu00795	day 14-15	0.000103	NB1-j	NB1-j_cl	NB1-j_or	NB1-j_fa	NB1-j_ge
Otu00741	day 14-15	0.000237	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	NK4A214_group
Otu00681	day 14-15	0.000127	Proteobacteria	Gammaproteobacteria	Burkholderiales	Neisseriaceae	Neisseriaceae_unclassified
Otu00246	day 14-15	0.000114	Crenarchaeota	Nitrososphaeria	Nitrososphaerales	Nitrososphaeraceae	Nitrososphaeraceae_ge
Otu00326	day 14-15	0.000103	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium
Otu02395	day 14-15	0.000646	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	Oscillospiraceae_unclassified
Otu00019	day 14-15	0.72689	Firmicutes	Clostridia	Oscillospirales	Oscillospirales_fa	Oscillospirales_ge
Otu01478	day 14-15	0.008768	Firmicutes	Clostridia	Oscillospirales	Oscillospirales_unclassified	Oscillospirales_unclassified
Otu00044	day 14-15	0.6433	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Paludicola
Otu00260	day 14-15	0.000105	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Pasteurella
Otu00006	day 14-15	0.000799	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Pasteurellaceae_unclassified
Otu00027	day 14-15	1.850294	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Pediococcus
Otu00487	day 14-15	0.000228	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcales-Tissierellales_fa	Peptoniphilus
Otu00157	day 14-15	0.005026	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Peptostreptococcaceae_unclassified
Otu00251	day 14-15	0.000105	Firmicutes	Negativicutes	Acidaminococcales	Acidaminococcaceae	Phascolarctobacterium

Table 3: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu00050	day 14-15	0.000237	Firmicutes	Bacilli	Bacillales	Planococcaceae	Planococcaceae_unclassified
Otu02585	day 14-15	0.000105	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella
Otu00108	day 14-15	0.000114	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_NK3B31_group
Otu00147	day 14-15	0.000239	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_UCG-003
Otu00745	day 14-15	0.000131	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotellaceae_unclassified
Otu00079	day 14-15	0.00154	Bacteroidota	Bacteroidia	Bacteroidales	Dysgonomonadaceae	Proteiniphilum
Otu00083	day 14-15	0.00012	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonadaceae_unclassified
Otu00131	day 14-15	0.00762	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
Otu00362	day 14-15	0.00054	Firmicutes	Bacilli	RF39	RF39_fa	RF39_ge
Otu01739	day 14-15	0.000287	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Rhizobiaceae_unclassified
Otu00098	day 14-15	0.000127	Bacteroidota	Bacteroidia	Bacteroidales	Rikenellaceae	Rikenellaceae_RC9_gut_group
Otu00053	day 14-15	0.000103	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Romboutsia
Otu00090	day 14-15	0.246823	Firmicutes	Clostridia	Oscillospirales	Ruminococcaceae	Ruminococcaceae_unclassified
Otu01093	day 14-15	0.00011	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	SN8
Otu02557	day 14-15	0.002491	Actinobacteriota	Actinobacteria	Pseudonocardiales	Pseudonocardaceae	Saccharopolyspora
Otu00020	day 14-15	0.008543	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	Staphylococcus
Otu00177	day 14-15	0.02367	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas
Otu00099	day 14-15	0.017244	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus
Otu00129	day 14-15	0.000347	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Succinivibrio
Otu00621	day 14-15	0.00012	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Syntrophococcus

Table 3: Continued

OTU	Sample	Abundance	Phylum	Class	Order	Family	Genus
Otu00022	day 14-15	0.303707	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Terrisporobacter
Otu01753	day 14-15	0.000331	Spirochaetota	Spirochaetia	Spirochaetales	Spirochaetaceae	Treponema
Otu00340	day 14-15	0.00012	Actinobacteriota	Actinobacteria	Actinomycetales	Actinomycetaceae	Trueperella
Otu00013	day 14-15	0.174247	Firmicutes	Bacilli	Erysipelotrichales	Erysipelotrichaceae	Turicibacter
Otu00043	day 14-15	0.002699	Proteobacteria	Gammaproteobacteria	Burkholderiales	Methylophilaceae	UBA6140
Otu00158	day 14-15	0.001006	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	UCG-005
Otu00137	day 14-15	0.000234	Firmicutes	Clostridia	Oscillospirales	Butyricicoccaceae	UCG-008
Otu00966	day 14-15	0.000131	Firmicutes	Clostridia	Oscillospirales	UCG-010	UCG-010_ge
Otu00007	day 14-15	0.000216	Firmicutes	Bacilli	Mycoplasmatales	Mycoplasmataceae	Ureaplasma
Otu01206	day 14-15	0.00011	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Veillonella
Otu00402	day 14-15	0.00011	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrionaceae_unclassified
Otu00179	day 14-15	0.000105	Verrucomicrobiota	Kiritimatiellae	WCHB1-41	WCHB1-41_fa	WCHB1-41_ge
Otu00058	day 14-15	0.00236	Proteobacteria	Gammaproteobacteria	Cardiobacteriales	Wohlfahrtiimonadaceae	Wohlfahrtiimonas
Otu00024	day 14-15	0.000127	Bacteroidota	Bacteroidia	Bacteroidales	p-251-o5	p-251-o5_ge
Otu00095	day 14-15	0.000131	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Selenomonadaceae	uncultured
Otu00472	day 14-15	0.000143	Actinobacteriota	Thermoleophilia	Gaiellales	uncultured	uncultured_ge

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