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Gut microflora development and impact on life-long health and performance.

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Executive Summary

A favourable gut microbiota is important for the optimal growth and performance of chicken, while an unfavourable microbiota may promote enteric infections, leading to decreased growth rates, poor feed conversion and increased mortality. The gut microbiota can influence the host's gastrointestinal development, biochemistry, immunology, physiology, and non-specific resistance to infection. Understanding the dynamics of the poultry gut microbial community is necessary to develop strategies to improve feed efficiency and growth rate, avoid intestinal disease and identify better feed additives and nutrient levels that influence beneficial microbial communities. The first two weeks post-hatch have been shown to be a dynamic period for gut microbiota changes in poultry. This also corresponds to a period of rapid development of the gastrointestinal tract (GI) and enteric immunity. In order to better understand the broiler gut microbiota and implications for animal health and production we investigated gut microbiota development and succession in the first two weeks post-hatch, as well as, determining the influence three in-feed antimicrobials (avilamycin, flavophospholipol and zinc bacitracin) had on normal gut microbiota development and colonisation.

In the first 17 days post-hatch both the ileal and caecal gut microbiota showed significant changes in bacterial community composition. A complex microbiota was already detectable at three days post-hatch with lactobacilli (*Lactobacillus johnsonii*, *L. reuteri* and *L. crispatus*) being dominant. Other bacteria characterised within the ilea and ceaca in the first 17 days post-hatch belonged to the phyla Firmicutes, Bacteroidetes and Proteobacteria, as well as unclassified bacteria. Many microbes are not easily culturable and DNA analysis has revealed a greater diversity. Some bacteria could be classified to the level of class (Bacilli), order (Clostridiales) or even family (*Lachnospiraceae, Lactobacillaceae, Enterobaceriaceae, Ruminococcaeceae, Bacteroidaceae* and *Oxalobacteraceae)*. In some cases sequence could be classified to the level of genera and included *Shigella*, *Lactobacillus* and *Lachnospiraceae Incertae Sedis*.

Age was found to influence gut microbiota development regardless of the dietary in-feed antimicrobial treatment. The ileal and caecal microbiota also developed differently with age. Caecal microbial communities took longer to stabilize than the ileal community. Similarity in ileal microbial community composition among the younger birds was generally lower than for the older birds. This indicated that the initial gut microbiota colonising the chick can be highly variable and that desirable microbiota colonisation could have great potential for optimal gut and enteric immunity development. The ileal and caecal microbial communities shared some common bacterial species but were generally distinct, suggesting that seeding of the distal GI occurred from the proximal GI with passage of digesta. However, most bacterial species had their own environmental niche within the GI tract.

In-feed antimicrobials altered the ileal and caecal gut microbial communities as early as 3 days post-hatch. In-feed antimicrobial response was greatest within the ileum and most predictable for avilamycin and flavophospholipol. The lack of consistent response to zinc bacitracin was inexplicable, but may have been due to resistant bacteria colonising the gut. No performance differences in response to the addition of in-feed antimicrobials were detected in the first 17 days post-hatch. This is not unusual for experiments undertaken in research facilities with high hygiene standards. Although the gut microbiota was influenced by in-feed antimicrobials, we can speculate that no detrimental bacteria colonised and/or dominated the gut microbiota in these birds which may have compromised performance.

We have previously shown that diet related changes in gut microbiota are linked to broiler performance (CRC 03-3a). In this study we also investigated a number of independent broiler performance trials to see if we could identify gut bacteria which are

consistently associated with broiler performance. The greatest determinant of resident gut microbiota is the host's diet. Although knowledge of the ideal gut microbiota is still incomplete, it is apparent that a variety of diets can equally support optimal bird performance and maintain a healthy gut microbial balance. Despite variations in the microbial composition of birds receiving differing dietary treatments we were able to identify nine operational taxonomic units (OTU; bacterial species or taxonomically related group of bacteria) which were common and related to differences in broiler performance across the three Australian feeding trials. These included OTU 180, 492, 564-566, 936-938 identified within the ileum and OTU 140-142, 218-220, 284-286, 312 and 482 identified within the caeca. OTU 564-566 was predominately associated with lower performance, while OTU 492, 140-142 and 482 were predominantly associated with improved performance.

The nine OTU identified may represent 22 different bacterial species or phylotypes. Some of these phylotypes were identifiable to the species level, however, the majority remained unclassified bacteria. Where bacteria were identifiable to the phyla level they belong predominantly to the Firmicutes and Bacteroidetes. Some bacteria could be classified to the level of order (Clostridiales), family (*Lachnospiraceae*, *Enterobacteriaceae*), genus (*Gallibacterium*, *Alistipes*, *Bacteroides*) or even species, with three *Lactobacillus* species implicated. The relative abundance of the Bacteroidetes and Firmicutes differed in a study of genetically predisposed obese versus lean mice, indicating particular bacterial groups have increased capacity for energy harvest. Although many of our potential performance related bacteria were unclassified, they did show high sequence similarity with those identified from studies investigating the relationship between the gut microbiome and host metabolic phenotype, innate immunity and gut microbiota, gut microbiota in various host species including poultry and the role of gut microbiota in gut health.

Quantitative PCR (qPCR) assays have been developed to five of these potential performance related phylotypes and it is possible to design assays to the remaining phylotypes. These putative performance related assay will need to be validated to prove whether or not they are true indicators of broiler performance. A vast amount of poultry gut bacterial phylogenetic information (post-hatch and performance related) has also been generated by this project which may aid in the development of other diagnostic platforms, such as a bacterial microarray chips. Furthermore, this project has also generated an extensive library of gut bacterial DNA from birds in various performance trials. This is valuable resource which could be utilised in future studies investigating aspects of gut microbiota and broiler performance. For example, it can be used to validate quantitative assays for performance related bacteria or to generate more detailed phylogenetic information on the bacterial communities present in performance related groups.

These results suggest that gut microbiota may be able to be manipulated immediately post-hatch. This may be via spray inoculation with a probiotic or synbiotic or even *in-ovo* feeding with a prebiotic at the hatchery. It may also be possible to isolate some of the post-hatch micro-organisms identified in this study for development of probiotic products. Identification and characterisation of potential performance related bacteria is an exciting finding but will need further development and validation. By obtaining a better understanding of the microbial balance it will be possible to further develop dietary strategies for managing the gut microbiota and broiler performance. Nutritional strategies to manage the composition of the intestinal microbiota and thus detrimental or beneficial outcomes will have practical value in the future and specific assays targeting performance related organisms may aid in diet or feed additive development.

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Introduction

The primary function of the gastrointestinal (GI) tract is to absorb nutrients from the diet and excrete waste products. During the first week post-hatch the growth of the chicken GI system far exceeds that of other organs in the body (Ferket, 2009). The optimum functioning of the GI tract is essential if the bird is to achieve its genetic potential. The GI tract also contains a unique microbial ecosystem which is affected by the flow of nutrients from the diet, host secretions and the systemic responses of the host (Rehman *et al.*, 2007). Any disturbance to the gut microbiota brought about by changes in diet composition, host immunity or gut physiology can lead to dysbacteriosis and/or enteritis. Hence, a favourable gut microbiota is important for the optimal growth and performance of chicken, while an unfavourable microbiota may promote enteric infections, leading to decreased growth rates and increased mortality.

The role of commensal gut microbiota in animal production has received much interest, particularly since the withdrawal of in-feed antimicrobials in the European Union (EU) in 2006. The use of in-feed antimicrobials in the poultry industry has played a major role in the control of pathogenic bacteria, and has had positive effects on animal welfare, animal production, economic return and food safety. However, the exact mode of action of antibiotics has not been determined. It is generally believed that antibiotics modulate gut microbiota and dampen immune response (Niewold, 2007). Many studies are now investigating potential alternatives to in-feed antibiotics, such as prebiotics, probiotics, essential oils and dietary acidifiers (Choct, 2009), yet a fundamental understanding of how these compounds influence the gut microbiota, immunity, health, physiology and ultimately production traits of the bird is lacking. Gut health has been a long neglected frontier because both the gut microbiota and the enteric immune systems have evaded understanding. Both are complex systems and the sensitive tools to investigate them fully have been lacking.

Gut microbiota positively influence the host's gastrointestinal development, biochemistry, immunology, physiology, and non-specific resistance to infection (Gordon and Pesti, 1971). The gastrointestinal microbiota has one of the highest bacterial cell densities of any ecosystem, and in poultry ranges from 10^7-10^{11} bacteria per gram gut content (Apajalahti *et al*., 2004). The collective microbial genome (microbiome) has a coding capacity that vastly exceeds that of the host's genome and encodes biochemical pathways that the host has not evolved (Egert *et al*., 2006). The gut microbiota of chicken has been reported to consume approximately a fifth of its host's daily dietary energy requirements, with the gut accounting for approximately 70 % of the bird's daily protein turnover (Ferket, 2009). The gut also has an important immunological function which is enhanced by contact with the intestinal microbiota and/or with immuno-modulating compounds in the feed (Klasing, 2007). Anaerobic metabolism of dietary compounds by the gut microbes produce short chain fatty acids (SCFA) and other metabolites which may either have beneficial or detrimental effects on the host. SCFA produced by the gut microbiota have been implicated in numerous host and microbe related activities; these include antibacterial properties, modulation of the bile and pancreatic secretions, supply of energy for epithelial cell proliferation, mucus production and gene expression (Rehman *et al*., 2007).

Changes in gut microbiota immediately post-hatch and the impact of dietary modification to increase productivity have not been investigated in great detail. This knowledge is essential if we wish to reduce reliance on in-feed antimicrobials, a major outcome sought in Australian Poultry CRC mark I. Until recently dietary modification strategies reported in the literature have been targeted well after hatch. We have shown that various dietary modifications introduced after two weeks of age lead to changes in gut microbiota composition and are linked to performance (CRC 03-3a; Torok *et al*., 2008), with specific bacteria having been correlated to performance. We have also shown the gut microbiota changes with age with the most dramatic changes evident within the first two weeks post-hatch. Identification of gut microbiota associated with performance traits will be useful to industry for assessing impact of feed and management on gut health and performance. Improvement in performance may be due to the presence of beneficial and/or absence of detrimental bacterial species.

Indeed, it has recently been shown that in genetically predisposed obese mice versus lean mice that the gut microbiota differ in relative abundance of the Bacteroidetes and Firmicutes (Turnbaugh *et al.,* 2006) indicating particular bacterial groups have increased capacity for energy harvest.

Objectives

A principal objective of the Poultry CRC is "sustainable production of chicken meat without reliance on antibiotics". Two key industry outcomes to be addressed within this particular project were:-

- Thorough understanding of the key factors influencing digestive function and gut microbiota of broiler chickens, maintaining efficient production without the use of antibiotics.
- Controlled microbial colonisation of the gut of newly hatched chickens to maintain a healthy gut microbiota throughout the productive life of the bird.

The specific aims of this project were:

(a) Identification of bacteria associated with improved performance traits in broilers.
(b) Development of diagnostic tests for indicator bacteria associated with broiler performance.
(c) Identification of bacterial components associated with early establishment of a healthy gut microbiota, which may be used to promote beneficial life-long colonisation.

Methodology

Commensal gut bacterial colonisation and succession were investigated in the first two weeks post-hatch under a controlled experiment at the Pig and Poultry Production Institute (PPPI), Roseworthy. Gut microbiota development was investigated for chicks on a control diet (no supplementation with in-feed antimicrobials) and with the inclusion of in-feed antimicrobials in the diet. This established a baseline for normal commensal microbiota development. Chick performance was monitored by live weight, feed consumption and feed conversion ratio. Overall ileal and caecal gut microbial communities were examined using the microbial profiling technique of terminal restriction fragment length polymorphism analysis (T-RFLP; Torok *et al*., 2008) which was developed in Poultry CRC project 03-3a. More detailed analysis of lactobacilli species within the ilea was done by Dr Gwen Allison (Australian National University) using Lactobacillus PCR denaturing gradient gel electrophoresis (Lac-PCR DGGE; Walter *et al*., 2001). Both T-RFLP and Lac-PCR DDGE rely on PCR amplification of the 16S subunit of the bacterial ribosomal RNA (rRNA) present in biological samples.

Gut bacterial species linked with poultry production traits were identified. Linkage was established with commercial research facilities and poultry research stations undertaking large-scale broiler growth studies. Linkage with three independent trials was made to maximise opportunities for obtaining gut samples from controlled experiments showing significant differences in broiler performance. Gut microbial communities from each experiment was examined by T-RFLP. Bacteria consistently associated with performance traits across trial were identified.

Important bacteria associated with post-hatch gut microbial colonisation and succession, as well as, potential performance related bacteria identified across feeding trials were characterised at the genome sequence level. Targeted cloning and sequencing of terminal restriction fragments (T-RF) from T-RFLP samples (Widmer *et al.,* 2006) made bacterial classification possible. Genome sequence information generated from potential performance related bacteria was used to design quantitative PCR (qPCR) assays making detection of these organisms both specific and quantitative.

Chapter 1: Influence of in-feed antimicrobials on post-hatch gut microbiota development in broiler chicks

Introduction

Knowledge of changes in gut microbiota immediately post-hatch and the impact of dietary modification are essential if we wish to reduce reliance on in-feed antimicrobials. Since 2006, when antibiotics were banned in the EU for growth promotion in poultry, much interest has focussed on the role of gut microbiota in animal health, production and product safety. In-feed antimicrobial modulate gut microbiota and dampen immune response (Niewold, 2007). The digestive system in chickens undergoes dramatic growth and transformation during the immediate post-hatch period in terms of bacterial colonisation and the demand to digest and absorb complex adult-type foodstuffs. These changes call for rapid adaptation of several physiological systems, including digestive capacity, barrier formation and development of protective immunity. Gut microbiota positively influence the host's gastrointestinal development, biochemistry, immunology, physiology, and non-specific resistance to infection (Gordon and Pesti, 1971). The initial microbiota to which chicks are exposed, as well as the nutrient composition of their diet, affect their commensal microbiota and the development of the immune system (Shira *et al.*, 2005).

Until recently dietary modification strategies reported in the literature have been targeted well after hatch. We have shown that various dietary modifications introduced after two weeks of age lead to changes in gut microbiota composition and are linked to performance (Torok *et al*., 2008). We have also shown the gut microbiota changes with age with the most dramatic changes evident within the first two weeks post-hatch. In this chapter immediate post-hatch commensal microbiota development will be identified and characterised in order to gain a better understanding of the optimal gut microbiota development under a variety of dietary conditions. By evaluating the effects of in-feed antimicrobials on post-hatch gut microbiota development and identifying key bacterial species this information may assist in the formulation of diets which facilitate beneficial microbial colonisation of the gastrointestinal tract. Such knowledge will aid in the development of alternatives to current in-feed antimicrobials in sustainable poultry production. The three in-feed antimicrobials (avilamycin, flavophospholipol and zinc bacitracin) investigated in this study were chosen because of their relevance to the Australian poultry industry and because they have been reported to have varying modes of action on bacteria *in vitro*.

The aims of this study were to characterise the normal gut microbiota development post-hatch; determine what influence in-feed antimicrobial have on gut microbiota development; and link improved performance, as a result of in-feed antimicrobials, to changes in gut microbiota.

Materials and methods

Birds and housing

All experimental work with animals was done at the Pig and Poultry Production Institute (PPPI), Roseworthy Campus, University of Adelaide with animal ethics approval from both the Department of Primary Industries and Resources of South Australia (PIRSA) and the University of Adelaide. Newly hatched (n = 640) vent sexed male broiler chicks (Cobb 500) were obtained from a local hatchery (Baiada Hatchery, Gawler, South Australia). On arrival chicks were weighed in groups of 40 and allocated to one of 16 raised floor pens (0.9 m x 1.8 m) within a climate controlled room. The floor of each pen was covered with brown paper and spread with fresh pine sawdust. Each pen also had its own feeder, drinker and brooding lamp for warmth. The experiment had a 4 x 4 randomised block design with four replicate pens receiving one of four diets from hatch (n=160/treatment). The four

experimental diets were based on a standard commercial starter diet (Steg 600 starter, Ridley Agriproducts, Australia) without any coccidiostats added and included: the commercial starter crumbles without addition of an antibiotic (control diet); control diet with addition of zinc bacitracin (50 ppm); control diet with addition of flavophospholipol (2 ppm); and control diet with addition of avilamycin (15 ppm).

Pen bird weights and feed consumed were recorded at days 1, 3, 5, 7, 10, 12, 14 and 17 post-hatch to allow weight gain and feed conversion ratio (FCR) to be calculated. FCR = pen weight gain (live + dead chicks)/feed consumed. Live weight was also recorded for individual chicks which were taken for microbial profiling.

Microbial profiling

Sample collection and nucleic acid extraction

At 3 and 5 days post-hatch, six chicks were taken from each of the 16 pens (n=24 birds/dietary treatment), while at 7, 10, 12, 14 and 17 days post-hatch three birds were taken from each of the 16 pens (n=12 birds/dietary treatment) for microbial profiling. Chicks were euthanized by cervical dislocation. Approximately a 2 cm section of the ileum (tissue and associated digesta), midway between the Meckel's diverticulum and caecal junction, as well as, both caeca were collected from each chick. Samples obtained from chicks aged 3 and 5 days were pooled due to limited quantity of material available from these young birds. From the six chicks taken per pen at 3-5 days of age gut samples were pooled n=2. Samples collected from birds 7-17 days post-hatch were not pooled. Care was taken not to cross contaminate samples. Following collection samples were kept on ice until frozen at -20^{o}C and then later freeze dried. Total nucleic acid was extracted from chicken gut by a modification (Torok *et al*., 2008) of a proprietary extraction method developed by the South Australian Research and Development Institute (Stirling *et al*., 2004).

T-RFLP

Terminal restriction fragment length polymorphism (T-RFLP) analysis was done following the technique described by Torok *et al*. (2008). Bacterial rRNA was amplified with universal 16S bacterial primers 27F (Lane, 1991) and 907R (Muyzer *et al*., 1995). The forward primer (27F) was 5′-labeled with 6-carboxyfluorescein (FAM) to enable subsequent detection of terminal restriction fragments (T-RFs). PCR reactions were done in duplicate in 50 µl volumes according to Torok *et al*., (2008). Following PCR all amplification products were quantified by fluorometry and duplicate PCRs which varied by less than 20% in fluorescein counts were pooled. Specificity of PCR products were analyzed by gel electrophoresis on a 1.5% agarose gel and visualized after staining with ethidium bromide. Approximately 200 ng PCR product was digested with 2 U *Msp*I (Genesearch, Arundel, Australia) in duplicate following manufacturer's instructions. The lengths of fluorescently labeled T-RFs were determined by comparison with an internal size standard (GeneScan 1200 LIZ; Applied Biosystems, Australia) following separation by capillary electrophoresis on an ABI 3730 automated DNA sequencer (Applied Biosystems, Australia). Data was analyzed using GeneMapper v3.7 software (Applied Biosystems, Australia). Data points generated by the GeneMapper software were further analyzed using a custom built database containing queries to validate data points and generate outputs for statistical analysis (Torok *et al*., 2008). The resulting fragments were treated as operational taxonomic units (OTU), representing particular bacterial species or taxonomically related groups of bacteria.

Lac-PCR DGGE

Lactobacillus specific PCR denaturing gradient gel electrophoresis (Lac-PCR DGGE) analysis was used to investigate the diversity of *Lactobacillus* species and related genera in the ilea. Group-specific lactobacillus primers, Lac1 and Lac2-GC, were used to amplify the V3 region of the 16S rRNA from total DNA using the Cool Gradient Palm Cycler 9600 (Corbett Research, Sydney, Australia). Pooled DNA was used as template. The pooled samples were prepared by combining the same amount of DNA from the ilea of bird taken from the same pen (n=3), as well as fed the same diet

(n=12) for each of the seven age groups investigated. The PCR products were subjected to DGGE (Lac-PCR DGGE) using the Bio-Rad DCode Universal Mutation Detection System (Hercules, California, USA) as outlined previously (Walter *et al*. 2001). Identification ladders for DGGE were prepared by combining the Lac-PCR products from DNA extracted from the reference strains (*Lactobacillus acidophilus*, *Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus johnsonii*, *Lactobacillus reuteri* and *Lactobacillus salivarius* subsp. *salivarius*, *Pediococcus acidilactici* and *Pediococcus pentosaceus*). *L. crispatus*, *Lactobacillus gallinarum* and *Lactobacillus amylovorous* belong to the Group A *acidophilus* taxonomic group, which cannot be distinguished using Lac-PCR DGGE (Guan *et al.* 2003), and will be referred to here collectively as LCGA. Gels were stained with ethidium bromide and viewed by UV transillumination. Lac-PCR DGGE band mobility was determined with the BioNumerics software package (Applied Maths, Sint-Martens-Latem, Belgium).

Statistical analysis

Performance data were analysed with SAS for Windows version 9.1 software package (Base SAS software; SAS Institute Inc., Cary, NC, USA). Univariate analysis of variance (ANOVA) was used to determine effects of block and in-feed antimicrobial (fixed factors) on bird performance, as measured by live weight, feed consumed and FCR, using the General Linear Model (GLM) with differences between treatments determined by Duncan's Multiple Range Test. ANOVA was also used to determine effects of in-feed antimicrobial on live weights of individual chicks taken for microbial profiling. Bird mortality was recoded for each pen and mean values ± SEM for each treatment calculated over the experimental period.

OTU obtained from the ileal and caecal contents of 240 individual broiler chicks aged 7 to 17 days and 96 pooled (n=2) samples from birds aged 3 and 5 days were analyzed using multivariate statistical techniques (PRIMER 6; PRIMER-E Ltd., Plymouth, UK). These analyses were used to examine similarities in chicken ileal and caecal bacterial communities associated with age and in-feed antimicrobial treatment. Bray-Curtis measures of similarity (Bray and Curtis 1957) were calculated to examine similarities between gut microbial communities of birds from the: T-RFLP data (following standardization and fourth root transformation); and the presence/absence Lac-PCR DGGE data (as scored against the reference *Lactobacillus* strains). One-way analysis of similarity (ANOSIM) (Clarke, 1993) was used to test if ileal or caecal microbial communities were significantly different between age groups and in-feed antimicrobial treatments. The *R*-statistic value describes the extent of similarity between each pair in the ANOSIM analysis, with values close to unity indicating that the two groups are entirely separate and a zero value indicating that there is no difference between the groups. Similarity percentages (SIMPER) (Clarke, 1993) analyses were done to determine the overall average similarity in ileal or caecal microbial community composition among birds fed the same diet and to determine possible OTU driving significant differences in bacterial community composition between treatments. Hierarchical cluster analysis (CLUSTER) (Clarke, 1993) was done to show changes in gut microbial communities associated with age.

Cloning and sequencing operational taxonomic units

Isolation of OTU of interest

A combination of adapter ligation, fragment size selection, and re-amplification with adapter specific PCR was used to isolate T-RFs of interest as described by Widmer *et al.* (2006). T-RFs are characterised by a specific PCR primer sequence (27F) at the 5'-end and a specific restriction site (*Msp*I) at the 3′-end. As this structure does not allow for direct re-amplification and further characterisation of a T-RF, a specific adapter matching the restriction site at the 3′-end of the T-RF and also containing a known PCR priming site is ligated to restriction digests from samples containing OTU of interest. Double-stranded *Msp*I-adapter was prepared and then ligated to restriction fragments as described Widmer *et al.* (2006). Size selection of T-RFs of interest was done by gel electrophoresis in a SEA 2000™ Electrophoresis Apparatus (Elchrom Scientific Inc., Switzerland) using precast Spreadex® gels (EL 400, 600, 800 or 1200; Elchrom Scientific Inc., Switzerland). Spreadex® gel type and electrophoresis conditions were chosen based on T-RF size range of interest and calculated using

the Gel Selection Guide and Virtual Electrophoresis Software (Elchrom Scientific Inc.). 10 µl ligation products were electrophoresed along with size standards 50 bp DNA Ladder (New England BioLabs) and GeneRuler™ 100 bp DNA Ladder (Fermentas) to allow size estimation. A size range of approximately ± 50 bp of the T-RFs of interest was excised from the gel. The gel slice was cut into equally sized pieces; each corresponding to a size range of approximately 12 bp. DNA was eluted from the gels as described by Widmer *et al*. (2006).

PCR amplification and cloning of isolated OUT

Eluted DNA was used as template for PCR amplification with primers 27F and *Msp*I-Adapter-primer (Widmer *et al.*, 2006). 6 µl of DNA template was amplified in a reaction volume of 30 µl containing 1 x PCR buffer (Applied Biosystems), 0.2 µM each primer, 2 mM $MgCl_2$, 0.8 mM dNTPs and 1 U AmpliTaq (Applied Biosystems). PCR amplification was done in a PTC-225 thermocycler (MJ Research) with initial denaturation for 5 min at 94°C, followed by 30 cycles with denaturation at 94°C for 45 s, annealing at 60°C for 60 s and extension at 72°C for 90 s and a final extension period at 72°C for 5 min. PCR products were analysed on a 2% agarose gel and visualised following staining with ethidium bromide. Single amplification products within expected size range were excised and purified using the NucleoSpin® Extract II kit (Macherey-Nagel) according to manufacturer's instructions. Purified products were ligated into pGEM®-T (Promega) and transformed into competent JM109 cells (Promega) following manufactures recommendations. Recombinant clones were identified by blue-white colour selection (Promega Technical Manual TM042) and confirmed by SP6/T7 PCR amplification of the plasmid insert. Recombinant clones were grown overnight in Luria broth containing ampicillin (Promega Technical Manual TM042). Plasmids were purified using the NucleoSpin® Plasmid kit (Macherey-Nagel) according to manufacturer's instructions.

16S rRNA sequence analysis

Plasmids were sequenced by Macrogen Inc., Korea. Vector sequence was removed using Pregap4 version 1.5 (Staden package) and aligned to identify consensus sequences using DNAMAN version 6 (Lynnon Corporation). Sizes of T-RFs were predicted *in-silico* using WatCut (University of Waterloo, Canada). Obtained 16S rRNA sequence data was assigned to a bacterial taxonomic hierarchy using Classifier (Ribosomal Database Project II Release 9). BLASTN (National Centre for Biotechnology Information; NCBI) was used to identify similarity with other sequences available in public genome sequence data bases. Bacterial classification and predicted *in-silco* TR-Fs for sequences obtained in this study are shown in Appendix B.

Results

Broiler performance

Bird performance, as measured by FCR, body weight and feed intake, was not influenced (P>0.05) by presence of in-feed antimicrobials in the diet in the first 17 days post-hatch (Table 1.1). Individual body weight of birds taken for microbial profiling was also not influenced (P>0.05) by use of in-feed antimicrobials (Table 1.2).

Table 1.1: Growth performance data of chicks during the first 17 days post-hatch.

Age (days)	Control	Zinc bacitracin	Flavophospho-lipol	Avilamycin	P value
	Live weight (g/bird)				
3	50.8 ± 0.3	50.4 ± 0.2	51.5 ± 0.4	50.1 ± 0.1	NS
5	66.0 ± 0.2	65.7 ± 0.6	65.6 ± 0.8	65.2 ± 0.6	NS
7	90.8 ± 0.9	90.1 ± 1.1	90.5 ± 1.7	90.1 ± 1.4	NS
10	122.4 ± 1.8	124.6 ± 0.7	126.6 ± 3.5	126.2 ± 2.2	NS
12	180.8 ± 2.8	188.0 ± 3.4	192.6 ± 7.5	192.6 ± 3.8	NS
14	242.0 ± 4.7	253.4 ± 3.2	254.0 ± 11.7	257.8 ± 6.2	NS
17	302.5 ± 10.3	318.4 ± 2.8	320.5 ± 15.0	323.0 ± 7.3	NS
	Feed intake (g/bird)				

3	18.1 ± 1.0	17.6 ± 0.7	16.9 ± 0.5	16.7 ± 0.5	NS
5	47.4 ± 0.8	48.0 ± 0.9	46.6 ± 1.3	47.0 ± 1.4	NS
7	95.4 ± 2.7	96.3 ± 1.1	95.5 ± 2.6	96.0± 2.9	NS
10	188.8 ± 6.8	198.8 ± 3.0	200.0 ± 7.6	198.1 ± 7.3	NS
12	287.4 ± 9.8	316.0 ± 5.7	310.5 ± 13.7	305.6 ± 8.1	NS
14	389.6 ± 9.0	445.4 ± 10.0	428.9 ± 22.1	433.1 ± 17.8	NS
17	596.8 ± 18.2	679.5 ± 10.1	681.4 ± 41.4	682.4 ± 34.7	NS
	FCR (g:g)				
3	1.20 ± 0.08	1.15 ± 0.03	1.20 ± 0.03	1.11 ± 0.01	NS
5	1.19 ± 0.02	1.21 ± 0.02	1.20 ± 0.02	1.18 ± 0.00	NS
7	1.34 ± 0.07	1.30 ± 0.02	1.27 ± 0.04	1.26 ± 0.04	NS
10	1.46 ± 0.07	1.45 ± 0.04	1.42 ± 0.06	1.39 ± 0.06	NS
12	1.50 ± 0.04	1.56 ± 0.02	1.55 ± 0.10	1.48 ± 0.07	NS
14	1.55 ± 0.05	1.66 ± 0.03	1.60 ± 0.10	1.59 ± 0.08	NS
17	1.61 ± 0.07	1.74 ± 0.04	1.70 ± 0.13	1.70 ± 0.13	NS

Live weight data are expressed as mean (g) ± SEM. Feed intake data are expressed as mean (g) ± SEM. FCR data are expressed as mean ± SEM. For all data n = 4 pens per treatment. NS is P>0.05.

Table 1.2: Body weight of chicks taken for microbial profiling.

Age	Control	Zinc bacitracin	Flavophospho-lipol	Avilamycin	P value
3	64.4 ± 1.2	65.7 ± 1.1	65.0 ± 1.0	63.5 ± 1.1	NS
5	92.0 ± 1.7	89.7 ± 1.7	91.0 ± 1.9	86.7 ± 1.8	NS
7	128.6 ± 3.9	135.2 ± 5.7	132.6 ± 4.0	128.0 ± 5.3	NS
10	193.7 ± 7.7	205.6 ± 7.3	214.8 ± 9.2	189.7 ± 6.8	NS
12	266.2 ± 10.1	274.3 ± 14.1	278.1 ± 16.3	262.0 ± 12.7	NS
14	321.1 ± 12.2	364.2 ± 10.5	327.9 ± 16.6	335.2 ± 12.0	NS
17	437.5 ± 26.0	432.8 ± 20.0	457.0 ± 20.9	471.1 ± 17.0	NS

Live body weight data are expressed as mean (g) ± SEM. For all data n = 4 pens per treatment. NS is P>0.05.

Overall chick mortalities in the first 17 days post-hatch ranged from 1.88% (zinc bacitracin group) to 5.63% (flavophospholipol group) (Table 1.3). All mortalities observed occurred between 3-10 days post-hatch.

Table 1.3: Chick mortality during first 17 days post-hatch.

Age (days)	Mortality (%)			
	Control	**Zinc bacitracin**	**Flavophospholipol**	**Avilamycin**
3	1.41 ± 0.20	0.00	1.88 ± 0.30	1.25 ± 0.18
5	0.70 ± 0.18	0.63 ± 0.16	3.75 ± 0.18	1.88 ± 0.16
7	0.70 ± 0.18	0.63 ± 0.16	0.00	0.00
10	1.41 ± 0.35	0.63 ± 0.16	0.00	0.00
12	0.00	0.00	0.00	0.00
14	0.00	0.00	0.00	0.00
17	0.00	0.00	0.00	0.00
Overall	**4.23 ± 0.12**	**1.88 ± 0.30**	**5.63 ± 0.47**	**3.13 ± 0.30**

Mortalities are expressed as percentage birds placed ± SEM. No other statistical analysis was done on this data. For all data n = 4 pens per treatment.

Changes in overall ileal and caecal bacterial communities

Age related changes

Within the ilea age influenced (P<0.05) microbial community composition regardless of dietary treatment (Table 1.4).

Table 1.4: One-way ANOSIM of ileal bacterial communities associated with age for each of the four dietary treatments. The R-statistic (above the diagonal) and significance level (below the diagonal; italics) are shown between pair wise comparisons. The R-statistic value describes the extent of similarity between each pair in the ANOSIM analysis, with values close to unity indicating that the two groups are entirely separate and a zero value indicating that there is no difference between the groups. Significance levels shown in bold were considered significant (P<0.05).

Diet	**Control (Global R=0.274, P=0.001)**							**Avilamycin (Global R=0.329, P=0.001)**						
Age	**3**	**5**	**7**	**10**	**12**	**14**	**17**	**3**	**5**	**7**	**10**	**12**	**14**	**17**
3		0.241	0.392	0.500	0.633	0.437	0.559			0.816	0.862	0.800	0.722	0.750
5	***0.012***		-0.037	0.059	0.314	0.192	0.311	***0.001***		0.032	0.101	0.082	0.106	0.164
7	***0.002***	*0.733*		0.087	0.444	0.315	0.451	***0.001***	*0.203*		0.241	0.213	0.237	0.440
10	***0.001***	*0.106*	*0.051*		0.219	0.176	0.266	***0.001***	*0.059*	***0.002***		0.008	0.050	0.060
12	***0.001***	***0.001***	***0.001***	***0.015***		0.089	0.244	***0.001***	*0.079*	***0.002***	*0.367*		0.047	0.165
14	***0.001***	***0.019***	***0.003***	***0.022***	*0.074*		0.053	***0.001***	***0.040***	***0.002***	*0.120*	*0.153*		0.117
17	***0.001***	***0.001***	***0.001***	***0.002***	***0.009***	*0.142*		***0.001***	***0.007***	***0.001***	*0.070*	***0.002***	***0.027***	
Diet	**Flavophospholipol (Global R=0.415, P=0.001)**							**Zinc Bacitracin (Global R=0.360, P=0.001)**						
Age	**3**	**5**	**7**	**10**	**12**	**14**	**17**	**3**	**5**	**7**	**10**	**12**	**14**	**17**
3		0.517	0.692	0.531	0.598	0.434	0.548		0.597	0.777	0.727	0.824	0.736	0.472
5	***0.001***		0.122	0.394	0.401	0.388	0.587	***0.001***		0.035	0.094	0.291	0.243	0.362
7	***0.001***	***0.018***		0.532	0.448	0.541	0.780	***0.001***	*0.191*		0.119	0.402	0.341	0.478
10	***0.001***	***0.001***	***0.002***		0.119	0.335	0.095	***0.001***	*0.050*	***0.029***		0.267	0.098	0.186
12	***0.001***	***0.001***	***0.001***	***0.028***		0.382	0.439	***0.001***	***0.001***	***0.001***	***0.001***		0.226	0.430
14	***0.001***	***0.001***	***0.001***	***0.001***	***0.001***		0.240	***0.001***	***0.004***	***0.001***	*0.057*	***0.002***		0.142
17	***0.001***	***0.001***	***0.001***	***0.036***	***0.001***	***0.003***		***0.001***	***0.001***	***0.001***	***0.022***	***0.001***	*0.054*	

Figure 1.1 shows the clustering of ileal microbial communities associated with age for birds raised on the control diet. Three main clusters were observed separating ileal microbial communities for bird aged 3-5 days, 5-12 days and 12-17 days. The cluster for birds aged 3-5 days was comprised of three sub-clusters and similarity in ileal microbial communities between these birds were generally lower than for the older birds.

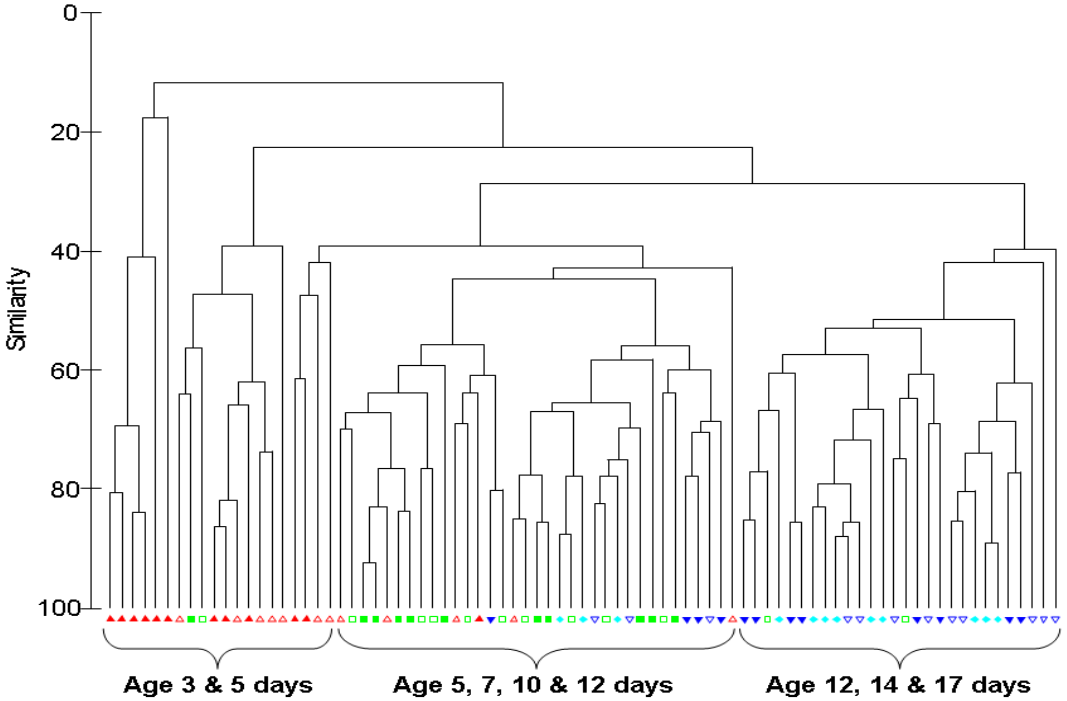

Figure 1.1: Dendogram representing relationships between T-RFLP profiles of ileal bacterial communities from individual birds at 3, 5, 7, 10, 12, 14 and 17 days post-hatch. All birds were raised on the control diet. ▲= 3 d, △= 5 d, ■ = 7 d, □ = 10 d, ▼ = 12 d, ▽ = 14 d and ◆= 17 d.

Age also influenced (P<0.05) the caecal microbial community composition (Table 1.5). Figure 1.2 shows the clustering of caecal microbial communities associated with age for birds raised on the control diet. Two main clusters were observed separating caecal microbial communities for bird aged 3 days and 5-17 days.

Table 1.5: One-way ANOSIM of caecal bacterial communities associated with age for each of the dietary treatments. The R-statistic (above the diagonal) and significance level (below the diagonal; italics) are shown between pair wise comparisons. Significance levels shown in bold were considered significant (P<0.05).

Diet	**Control (Global R=0.362, P=0.001)**							**Avilamycin (Global R=0.500, P=0.001)**						
Age	**3**	**5**	**7**	**10**	**12**	**14**	**17**	**3**	**5**	**7**	**10**	**12**	**14**	**17**
3		0.647	0.695	0.683	0.715	0.812	0.539		0.641	0.885	0.900	0.931	0.942	0.724
5	***0.001***		0.031	0.294	0.151	0.470	0.182	***0.001***		0.152	0.428	0.501	0.667	0.209
7	***0.001***	*0.239*		0.277	0.181	0.522	0.331	***0.001***	***0.033***		0.321	0.357	0.54	0.173
10	***0.001***	***0.002***	***0.003***		0.016	0.180	0.271	***0.001***	***0.001***	***0.001***		0.083	0.288	0.312
12	***0.001***	***0.015***	***0.009***	*0.338*		0.139	0.198	***0.001***	***0.001***	***0.001***	*0.062*		0.224	0.326
14	***0.001***	***0.001***	***0.001***	***0.005***	***0.029***		0.319	***0.001***	***0.001***	***0.001***	***0.002***	***0.002***		0.482
17	***0.001***	***0.014***	***0.002***	***0.001***	***0.007***	***0.001***		***0.001***	***0.006***	***0.009***	***0.001***	***0.001***	***0.001***	
Diet	**Flavophospholipol (Global R=0.572, P=0.001)**							**Zinc Bacitracin (Global R=0.542, P=0.001)**						
Age	**3**	**5**	**7**	**10**	**12**	**14**	**17**	**3**	**5**	**7**	**10**	**12**	**14**	**17**
3		0.927	0.943	0.889	0.870	0.905	0.787		0.797	0.968	0.950	0.883	0.978	0.706
5	***0.001***		0.021	0.549	0.444	0.835	0.312	***0.001***		0.329	0.317	0.54	0.614	0.136
7	***0.001***	*0.268*		0.599	0.465	0.818	0.379	***0.001***	***0.001***		0.327	0.263	0.649	0.481
10	***0.001***	***0.001***	***0.001***		0.117	0.282	0.477	***0.001***	***0.001***	***0.001***		0.274	0.184	0.348
12	***0.001***	***0.001***	***0.001***	***0.010***		0.413	0.456	***0.001***	***0.001***	***0.003***	***0.005***		0.279	0.412
14	***0.001***	***0.001***	***0.001***	***0.001***	***0.001***		0.570	***0.001***	***0.001***	***0.001***	***0.011***	***0.002***		0.421
17	***0.001***	***0.001***	***0.001***	***0.001***	***0.001***	***0.001***		***0.001***	***0.007***	***0.001***	***0.001***	***0.001***	***0.001***	

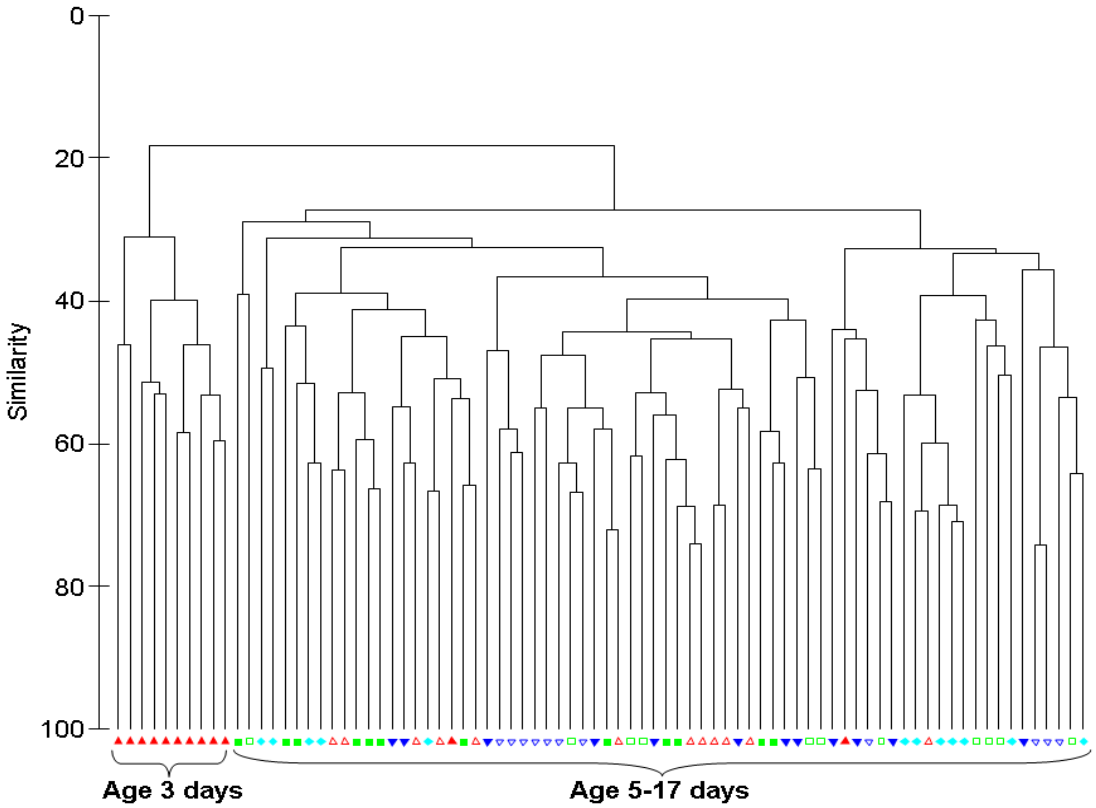

Figure 1.2: Dendogram representing relationships between T-RFLP profiles of caecal bacterial communities from individual birds at 3, 5, 7, 10, 12, 14 and 17 days post-hatch. All birds were raised on the control diet. ▲= 3 d, △= 5 d, ■ = 7 d, □ = 10 d, ▼ = 12 d, ▽ =14 d and ◆ = 17 d.

SIMPER analysis showed that the ileal microbial community similarity among chicks on the same dietary treatment ranged from 29-61% within the first 17 days post-hatch. Ileal bacterial community similarity was lower for the control group from 3 to 7 days post-hatch (29-50%) than for the avilamycin (43%-55%), flavophospholipol (45-67%) and zinc bacitracin (38-58%) groups. Within the caeca microbial community similarity among chicks on the same dietary treatment was lower and ranged from 34-59% in the first 17 days post-hatch. Chicks aged 3-5 days had a lower caecal microbial community similarity within the avilamycin (35-39%) and control (35-43%) groups as compared with the flavophospholipol (50-52%) and zinc bacitracin (50-59%) groups. However, by 17 days post-hatch all treatments had a comparable caecal microbial community similarity of 37-42%.

OTU (bacterial species or taxonomically related groups of bacterial) characterising the ilea bacterial communities in the first 17 days post-hatch regardless of dietary treatment were 60, 82, 86, 152, 176, 178, 180, 186, 188, 210, 212, 284, 286, 518, 560, 562, 566, 894, 932 and 938. OTU characterising the caeca within the first 17 days post-hatch regardless of dietary treatment were 78, 80, 82, 140, 142, 144, 176, 148, 178, 180, 198, 200, 216, 220, 286, 288, 296, 310, 312, 476, 480, 484, 490, 492, 566 and 600. Although there was some overlap in bacterial community composition (OTU 82, 178, 180, 286 and 566) between the two gut sections, on the whole each gut section exhibited a distinct microbial community.

In-feed antimicrobial related changes

In-feed antimicrobial treatments influenced ($P<0.05$) both the ileal and caecal microbial communities within the first 17 days post-hatch (Table 1.6). Influence of in-feed antimicrobials was most evident on the ileal microbial communities. Ileal microbial communities of chicks fed diets containing flavophospholipol were significantly different to those birds fed the control diet at all seven age groups investigated. Zinc bacitracin had the least consistent effect on gut microbial communities as compared with the control group, with differences only detected at 3 and 7 days within the caeca and 12 and 14 days within the ilea.

Where significant differences were detected between the control diet and those containing in-feed antimicrobials the OTU associated with the difference were identified in either the ilea (Table 1.7) or caeca (Table 1.8). These OTU represent bacterial species or related groups of bacteria and the differences detected between dietary treatments tend to related to relative abundance.

Table 1.6: One-way ANOSIM of the ileal or caecal microbiota associated with dietary treatments as investigated for ages 3-17 days post-hatch.

Age (days)	Ilea	Caeca
3	**Global R = 0.182, P = 0.001** Control vs Avilamycin * Avilamycin vs Flavophospholipol Control vs Flavophospholipol Flavophospholipol vs Zinc bacitracin	**Global R = 0.054, P = 0.039** Control vs Zinc bacitracin
5	**Global R = 0.073, P = 0.036** Avilamycin vs Flavophospholipol Control vs Flavophospholipol Flavophospholipol vs Zinc bacitracin	**Global R = 0.272, P = 0.001** Control vs Avilamycin Avilamycin vs Flavophospholipol Avilamycin vs Zinc bacitracin Control vs Flavophospholipol Flavophospholipol vs Zinc bacitracin
7	**Global R = 0.076, P = 0.008** Control vs Flavophospholipol Flavophospholipol vs Zinc bacitracin	**Global R = 0.117, P = 0.002** Control vs Flavophospholipol Control vs Zinc bacitracin Flavophospholipol vs Zinc bacitracin
10	**Global R = 0.067, P = 0.022** Avilamycin vs Zinc bacitracin Control vs Flavophospholipol Flavophospholipol vs Zinc bacitracin	**Global R = 0.088, P = 0.014** Avilamycin vs Zinc bacitracin Flavophospholipol vs Zinc bacitracin Avilamycin vs Flavophospholipol

12	**Global R = 0.187, P = 0.001** Control vs Avilamycin Control vs Zinc bacitracin Control vs Flavophospholipol Flavophospholipol vs Zinc bacitracin	**Global R = 0.087, P = 0.005** Avilamycin vs Flavophospholipol Avilamycin vs Zinc bacitracin Flavophospholipol vs Zinc bacitracin
14	**Global R = 0.153, P = 0.003** Control vs Avilamycin Control vs Zinc bacitracin Control vs Flavophospholipol Flavophospholipol vs Zinc bacitracin	**Global R = 0.040, P = 0.102**
17	**Global R = 0.222, P = 0.001** Control vs Avilamycin Avilamycin vs Zinc bacitracin Control vs Flavophospholipol Flavophospholipol vs Zinc bacitracin	**Global R = 0.170, P = 0.001** Control vs Avilamycin Avilamycin vs Flavophospholipol Avilamycin vs Zinc bacitracin Control vs Flavophospholipol Flavophospholipol vs Zinc bacitracin

* Significant pair wise comparisons identified (P<0.05)

Table 1.7: OTU contributing significantly to differences in ileal microbial communities between chicks on the control and listed in-feed antimicrobial diets.

Age (days)	Significantly different to control diet	Discriminating OTU	
		Control	**Antimicrobial**
3	Avilamycin	220	60, 86, 492, 518, 536, 560/562
	Flavophospholipol	220	86, 180, 186, 492, 518, 560, 566
5	Flavophospholipol	186, 574	176, 180, 210/212, 566, 938
7	Flavophospholipol	186/188, 574	178, 170, 184, 210, 248,
10	Flavophospholipol	186, 574	178/180, 212, 936
12	Avilamycin	186, 284/286, 936	152, 176, 212, 574
	Flavophospholipol	186, 284/286, 894	152,170, 176/178, 212, 936
	Zinc bacitracin	78, 86, 186, 284/286, 894	152, 176/178, 180, 212
14	Avilamycin	86, 284/286, 574, 894	70, 180, 566
	Flavophospholipol	86, 284/286, 894	70, 80, 180, 518, 566
	Zinc bacitracin	86, 284/286, 574, 894	70, 178/180, 566
17	Avilamycin	86, 186/188, 284/286, 574, 894, 936	70, 178/180, 566
	Flavophospholipol	86, 186/188, 284/286, 574, 894	70, 178/180, 566, 936

Table 1.8: OTU contributing significantly to differences in caecal microbial communities between chicks on the control and listed in-feed antimicrobial diets.

Age (days)	Differences between listed AGP and control	Discriminating OTU	
		Control	**AGP treatment**
3	Zinc bacitracin	220	198, 910
5	Avilamycin	140/142, 180, 286/288, 296, 482	-
	Flavophospholipol	140/142, 286/288, 290, 296, 480/482, 564	178/180, 200, 216/218, 476
7	Flavophospholipol	140/142	178/180, 198/200, 286, 476, 520, 564
17	Avilamycin	78, 144/146, 284/286, 296/298, 482,536, 910	68, 140/142, 198, 216/218, 520
	Flavophospholipol	78, 140/142, 144/146, 284/286, 536, 910,	216, 294, 310, 490,

Figure 1.3 shows the 12 individual ileal T-RFLP profiles which have been overlaid one on top of another to create a composite T-RFLP profile for each of the in-feed antimicrobial treatments shown to be significantly different to the control diet at 3 days of age. Peak position represents bacterial species and/or related groups of bacterial and is identified as an OTU. Peak height is semi-

quantitative and represents the amount of a particular OTU within the bacterial population. Figure 1.3 shows the diverse bacterial population already established at 3 days post-hatch, as well as, differences between the in-feed antimicrobials and control treatments. Although this figure does not show the inter-individual variation in bacterial profiles present within a treatment it does represent some of the statistical observations made in Table 1.7. For examples OTU 220 is predominantly associated with the control group. More importantly it shows that differences are not generally due to presence or absence of particular OTU in a treatment but more likely to be due to changes in abundance of common OTU. Changes in common OTU abundance is also noticeable with age, for example OTU 492-574 become less abundance at 17 days post-hatch (Figure 1.4) than at 3 days post-hatch (Figure 1.3). At 17 days post-hatch it is also observed that difference between the control treatment and the in-feed antimicrobials can be partially accounted for by and increased abundance of OTU 86, 286 and 894 in the control group and OTU 70 in the avilamycin and flavophospholipol groups. This is consistent with data presented in Table 1.7.

Figure 1.5 graphically represents the differences in caecal microbial profile between the control and zinc bacitracin groups. OTU 220 is more prevalent in the control groups as indicated in Table 1.8. Furthermore, apart from a few potential common OTU (186, 218/220, 492, 560, 574) the caecal microbial profiles of 3 day old broilers (Figure 1.5) is different to that of the ileal microbial profiles (Figure 1.3). By 17 days post-hatch the caecal microbial communities (Figure 1.6) are more diverse than that found within the ilea (Figure 1.4).

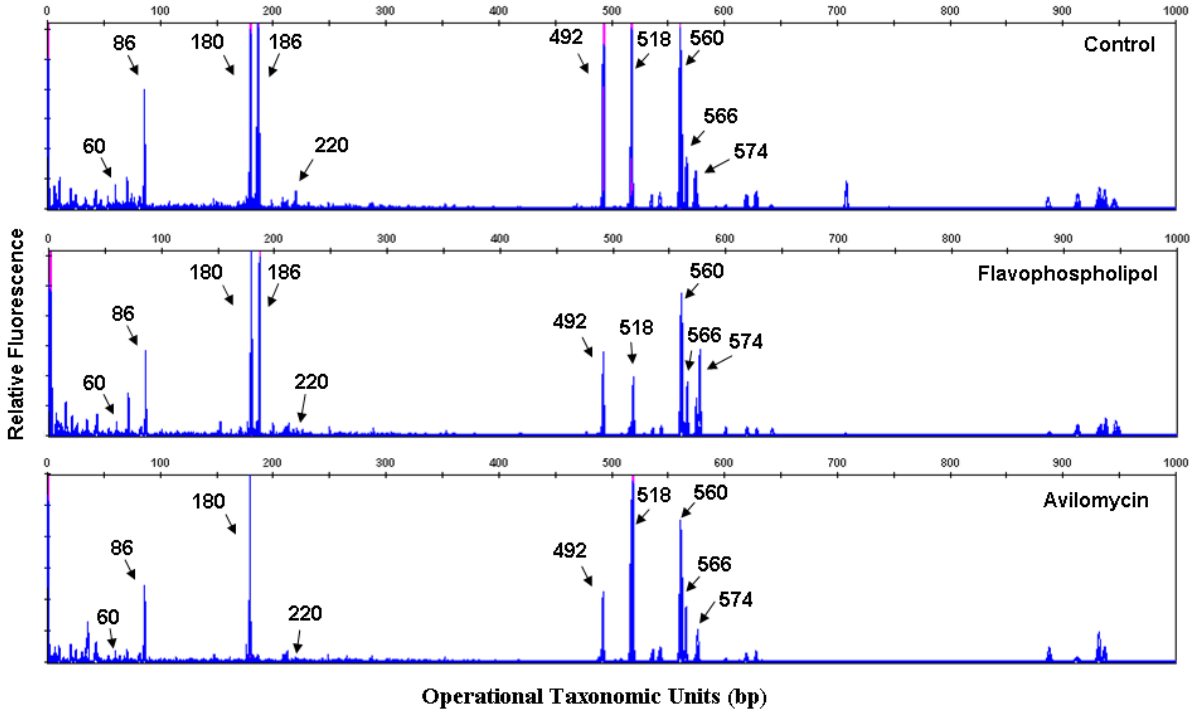

Figure 1.3: Comparison of composite T-RFLP profiles (n=12) for in-feed antimicrobial treatments showing significant differences in ileal bacterial communities to that of the control group at 3 days post-hatch.

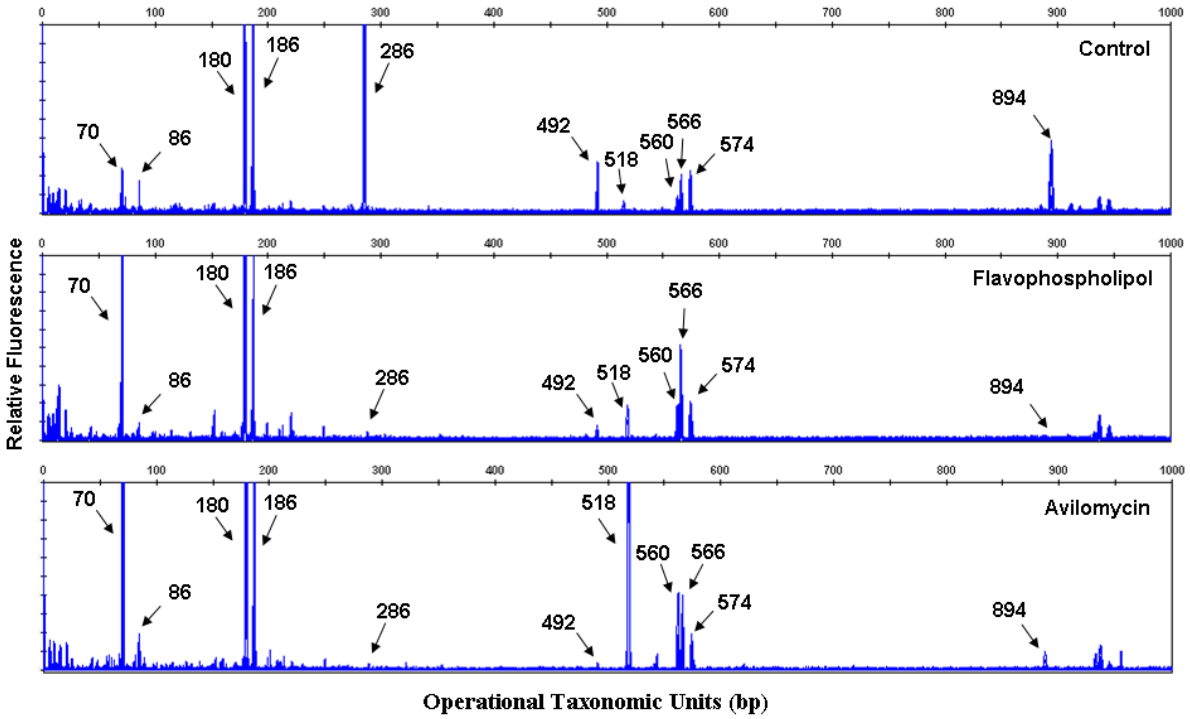

Figure 1.4: Comparison of composite T-RFLP profiles (n=12) for in-feed antimicrobial treatments showing significant differences in ileal bacterial communities to that of the control group at 17 days post-hatch.

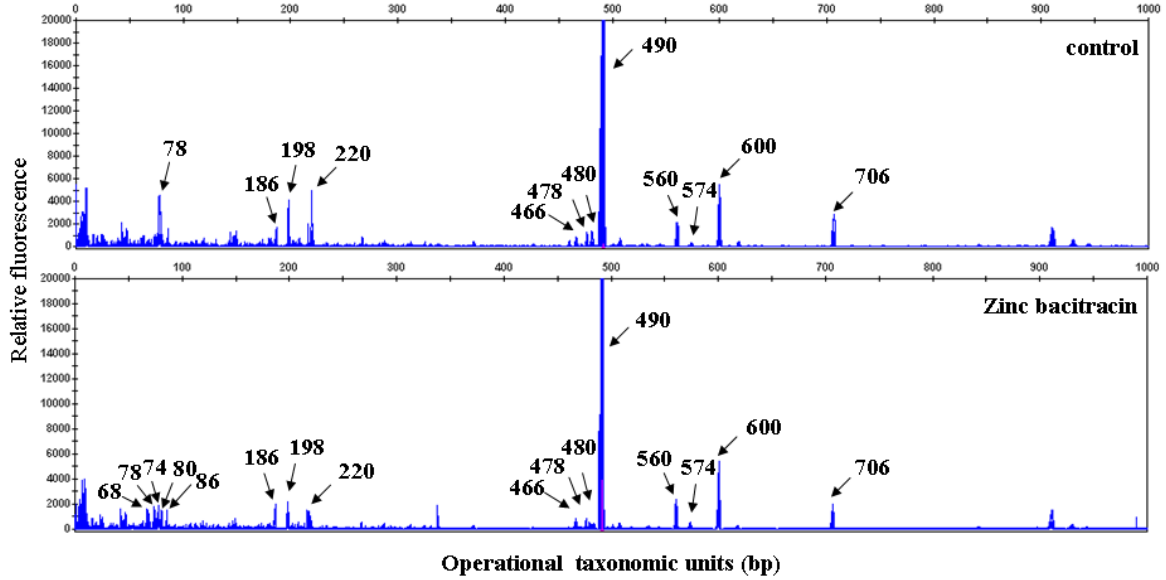

Figure 1.5: Comparison of composite T-RFLP profiles (n=12) for in-feed antimicrobial treatments showing significant differences in caecal bacterial communities to that of control group at 3 days post-hatch.

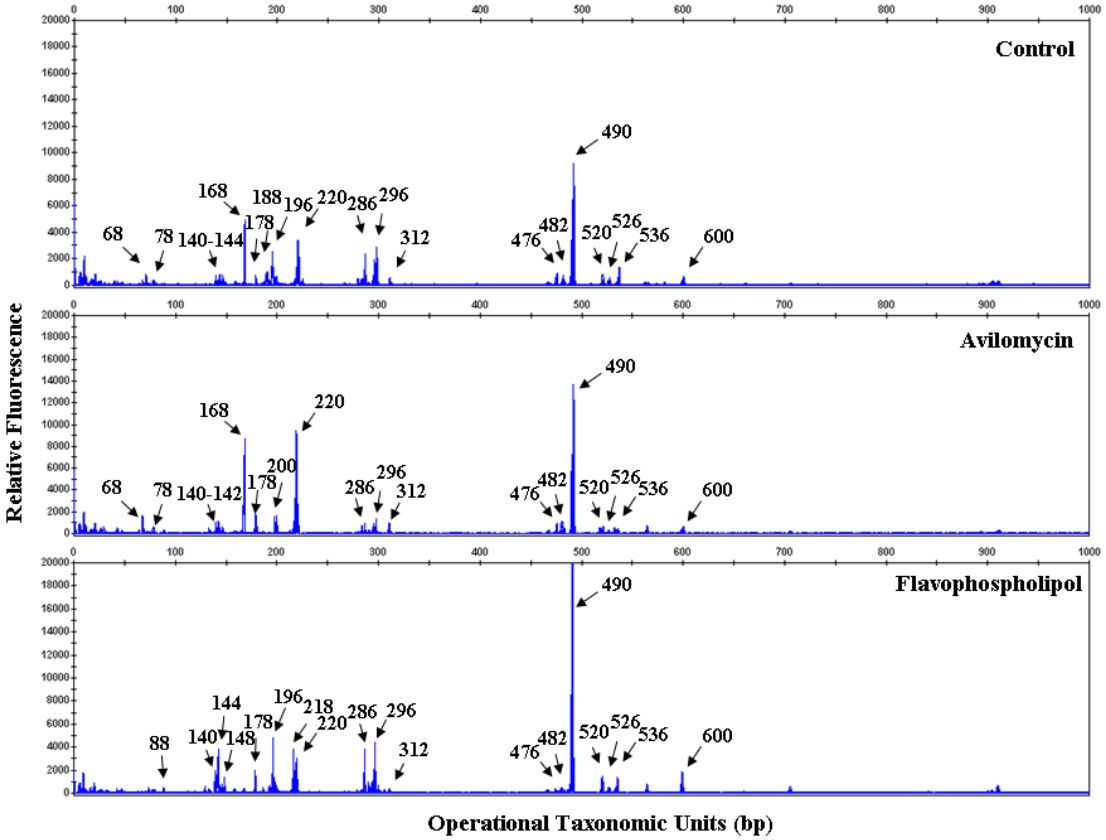

Figure 1.6: Comparison of composite T-RFLP profiles (n=12) for in-feed antimicrobial treatments showing significant differences in caecal bacterial communities to the control group at 17 days post-hatch.

Changes in ileal lactobacilli communities

In-feed antimicrobials did not significantly (P>0.05) influence the presence of lactobacilli within the ilea. However, age of birds did significantly alter Lactobacilli profiles. Significant differences were detected between age groups for each of the dietary treatments except within the control (Table 1.9).

Table 1.9: One-way ANOSIM of ileal lactobacilli communities associated with age for each of the dietary treatments. The R-statistic (above the diagonal) and significance level (below the diagonal; italics) are shown between pair wise comparisons. Significance levels shown in bold were considered significant (P<0.05).

Diet	**Control (Global R=0.070, P=0.121)**						
Age	**3**	**5**	**7**	**10**	**12**	**14**	**17**
3		-	-	-	-	-	-
5	-		-	-	-	-	-
7	-	-		-	-	-	-
10	-	-	-		-	-	-
12	-	-	-	-		-	-
14	-	-	-	-	-		-
17	-	-	-	-	-	-	

Diet	**Avilamycin (Global R=0.286, P=0.001)**						
Age	**3**	**5**	**7**	**10**	**12**	**14**	**17**
3		0.161	0.406	0.411	0.443	0.547	0.203
5	*0.114*		0.313	0.318	0.339	0.536	0.161
7	***0.029***	***0.029***		-0.302	0.214	0.146	0.375
10	***0.029***	*0.057*	*0.943*		0.063	0.307	0.406
12	***0.029***	*0.057*	*0.143*	*0.343*		0.589	0.563
14	*0.057*	*0.057*	*0.171*	*0.114*	***0.029***		-0.010
17	*0.143*	*0.200*	***0.029***	*0.057*	***0.029***	*0.486*	

Diet	**Flavophospholipol (global R=0.141, P=0.050**						
Age	**3**	**5**	**7**	**10**	**12**	**14**	**17**
3		0.219	0.589	0.651	0.589	0.323	0.245
5	*0.171*		0.255	-0.005	0.333	-0.089	-0.031
7	***0.029***	*0.086*		0.016	-0.016	0.141	0.115
10	***0.029***	*0.543*	*0.457*		-0.161	-0.323	0.068

Diet	**Zinc Bacitracin (Global R=0.312, P=0.001)**						
Age	**3**	**5**	**7**	**10**	**12**	**14**	**17**
3		0.037	0.463	0.880	0.981	0.963	0.259
5	*0.400*		0.042	0.349	0.563	0.130	0.266
7	*0.057*	*0.400*		-0.052	0.073	-0.016	0.234
10	***0.029***	***0.029***	*0.657*		0.073	0.208	0.458

12	***0.029***	*0.086*	*0.571*	*0.857*		-0.068	0.214	***0.029***	***0.029***	*0.257*	*0.400*		0.526	0.432
14	*0.057*	*0.600*	*0.229*	*1.000*	*0.657*		-0.094	***0.029***	*0.229*	*0.629*	*0.114*	***0.029***		0.401
17	*0.114*	*0.486*	*0.257*	*0.314*	*0.143*	*0.657*		*0.200*	*0.114*	*0.229*	***0.029***	*0.057*	*0.057*	

SIMPER analysis showed as birds grew older the prevalence of *L. johnsonii* and *L. reuteri* increased. *Pediococcus acidilactici* was only detectable in chicks at three days of age (Figure 1.7). Birds of all age groups contained the LCGA (Group A *acidophilus* taxonomic group) which could comprise *L. crispatus*, *L. gallinarum* and/or *L. amylovorous* and cannot be distinguished using the Lac-PCR DGGE technique.

Characterisation of post-hatch gut microbiota development

Targeted cloning and sequencing of OTU ranging from 60-600 bp generated 16 rRNA sequences information from 367 clones. Sequences were classified with a confidence threshold of 80% according to the hierarchy of domain, phylum, class, order, family, genus and species. Most of the sequences of cloned OTU could only be classified as unidentifiable bacteria. However, where sequences could be classified to the level of phyla they belonged to the Firmicutes, Bacteroidetes and Proteobacteria. Some sequences could be further classified to the level of class (Bacilli), order (Clostridiales) or even family (*Lachnospiraceae*, *Lactobacillaceae*, *Enterobaceriaceae*, *Ruminococcaeceae*, *Bacteroidaceae* and *Oxalobacteraceae)*. In some cases sequence could be classified to the level of genera and included *Shigella*, *Lactobacillus* and *Lachnospiraceae Incertae Sedis*. Three species of *Lactobacillus* were identified which could be matched to OTU determined as contributing to difference between the in-feed antimicrobial and control treatments.

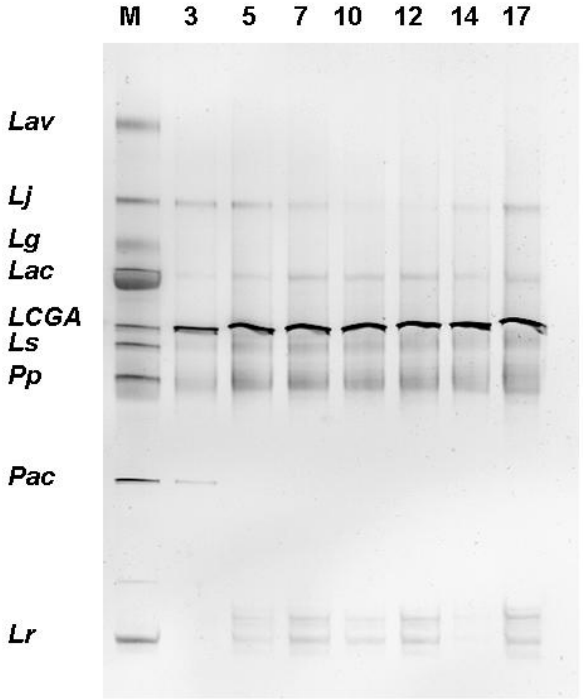

Figure 1.7: Lac-PCR DGGE analysis of pooled samples (n=12) for birds on the flavophospholipol treatments analysed at 3-17 days post-hatch. *Lactobacillus avarius* (Lav), *L. acidophilus* (Lac), *L. crispatus/L. gallinarum* and/or *L. amylovorous* (LCGA), *L. gasseri* (Lg), *L. johnsonii* (Lj), *L. reuteri* (Lr), *L. salivarius* subsp. *salivarius* (Ls), *Pediococcus acidilactici* (Pac) and *P. pentosaceus* (Pp).

Discussion

The in-feed antimicrobials avilamycin, flavophospholipol or zinc bacitracin did not improve broiler performance, as measured by live body weight, feed consumption or FCR, in the first 17 days

post-hatch. Overall chick mortalities were lowest for the zinc bacitracin group (1.9%) and highest for the flavophospholipol group (5.6%). The lack of significant performance responses in this study is not entirely surprising given the small number of experimental units (n=4 pens/treatment), and the fact that a growth promotion response to in-feed antimicrobials is not always evident in a highly sanitized research facility environment (Dumonceaux *et al.,* 2006). In a previous study, Pedroso *et al.* (2006) only noted significant performance differences in birds fed antibiotics when raised in floor pens as opposed to battery cages (where they were not exposed to litter or coprophagy), further supporting the importance of environment on in-feed antimicrobial response.

Our results show that the composition of the ileal and caecal microbiota changes with age during the period 3-17 days post-hatch regardless of dietary treatment. Within the ilea three major shifts in microbial community composition were observed and occurred at 3-5 days, 5-12 days and 12-17 days. The cluster for birds aged 3-5 days was comprised of three sub-clusters and the similarity in ileal microbial communities between these birds were generally lower than for the older birds. This shows that the initial gut microbiota colonising chicks can be highly variable. Within the caeca two major shifts in microbial community composition were observed at 3 days and 5-17 days. This may indicate that the caecal microbial community takes longer to stabilize than the ileal microbiota. Consistent with our observations, Hume *et al*. (2003) also showed shifts in caecal microbiota at 2 days and 5-20 days of age. Although some OTU were common to both the ilea and caeca, on the whole the caecal microbial communities were different to the ilea. Differences between the caecal and ileal communities related to the presence/absence of unique bacterial species and also to the relative contribution of ubiquitous bacteria. In contrast Lu *et al.* (2003) found that ileal and caecal microbiota were not significantly different at 3 days post-hatch and that the caecal microbiota were a subset of the ileal microbial community for the first 14 days post-hatch. The common OTU we observed within both the ilea and ceaca may suggest that the ileal microbial community does initially seed caecal colonisation; however, the two different environmental niches develop their own community structure with time.

Bacterial species (OTU 180 and 186) which appeared to be present in high numbers for all birds aged 3-17 days within both the ilea and caeca appear to represent *Lactobacillus* species. This is supported by evidence that Lactobacilli were detected in the ilea throughout this study using Lac-PCR DGGE. Most of the lactic acid bacteria reference strains were detectable in chicks 3 to 17 days of age. *P. acidilactici* was only detectable in birds aged 3 days and *L. johnsonii* and *L. reuteri* were more prevalent in the older chicks. The LCGA group was dominant in all age groups and the presence of *L. crispatus* was confirmed by 16S rRNA genome sequence information. These data support the autochthonous nature of these species in the chicken gastrointestinal tract, which have been reported to be present in birds of various ages (Knarreborg *et al*. 2002b; Lu *et al*., 2003; Gong *et al.*, 2008; Guan *et al.* 2003).

In addition to the influence that age and gut development had on the post-hatch ileal and caecal microbiota development, significant differences were detected in response to the various in-feed antimicrobials examined in this study. Two of the in-feed antimicrobials (avilamycin and flavophospholipol) had significant effects on both the ileal and caecal microbial communities although more consistently within the ileum. The proximal gut microbiota has been reported to be more susceptible to antibiotics than the distal gut (Dumonceaux *et al.,* 2006; Wise and Siragusa, 2007). In this study zinc bacitracin was not as consistent in its effects on either the ileal or caecal microbial communities. Why a greater affect was not detected with the zinc bacitracin is not known, however a possibility could be that the bacteria colonising the young chicken gut may have been carrying resistance genes to this antibiotic. Bacterial resistance to in-feed antimicrobials, including zinc bacitracin, has been previously reported. Resistant *Enterococcus faecalis, E. faecium,* staphylococci and lactobacilli have been identified in broiler flocks from hens which had received zinc bacitracin as a growth promotant, but which had never been feed to their offspring (Frei *et al*., 2001).

Lac-PCR DDGE did not detect differences in lactobacilli communities associated with in-feed antimicrobials, whereas T-RFLP not only identified differences in overall gut microbial communities associated with in-feed antimicrobial treatment but also identified species of the lactobacilli driving these differences. Both techniques have the ability to detect these particular *Lactobacillus* species,

however the power of statistical analysis on the Lac-PCR DGGE data was likely reduced as samples were pooled (n=3) and only had 4 replicates per treatment. We would suggest future analysis be done on individual samples with adequate replication ($n \geq 12$).

Most of the OTU (bacterial groups or taxonomically related groups of bacteria) characterised at the genome level belonged to unidentified bacteria, however where phyla could be identified they belonged to Firmicutes, Bacteroidetes and Proteobacteria. Some could be further classified to the family level (*Lachnospiraceae*, *Lactobacillaceae*, *Enterobaceriaceae*, *Ruminococcaeceae*, *Bacteroidaceae* and *Oxalobacteraceae)*. In a few cases sequences could be classified to the genus level and included *Shigella*, *Lactobacillus* and *Lachnospiraceae Incertae Sedis*. Three *Lactobacillus* species were identified which were shown to be influenced by in-feed antimicrobial treatment. Although most of the bacteria were unidentifiable they did show genome sequence similarity to other gastrointestinal inhabiting bacteria available in public genome sequence databases. The 16S rRNA genome sequence information generated in this study could be invaluable for developing targeted diagnostic approaches in gaining a better understanding of the gut microbiota in poultry health and production.

In conclusion we have shown that age and in-feed antimicrobials affect the composition of the total bacterial and lactobacilli communities. Furthermore, we have characterised bacterial species from in-feed antimicrobial treated communities. This may aid in a more targeted approach to investigating shifts in the gut microbiota in the future. Ultimately the knowledge of what constitutes a normal microbiota will aid in the development of viable alternatives to dietary antibiotics. In-feed antibimicrobials have been previously reported to influence the intestinal microbial communities, including *Lactobacillus* species (Dumonceaux *et al.*, 2006; Engberg *et al.*, 2000; Knarreborg *et al.*, 2002b; Pedroso *et al.*, 2006; Wise and Siragusa 2007; Zhou *et al*., 2007). However, most studies investigating effect of in-feed antimicrobials on gut microbiota have generally been done two weeks post-hatch or much later. Newly hatched chicks lack digestive microbiota and are highly susceptible to enteropathogen colonisation and infections (Hume *et al*., 2003). Bacterial communities have been identified in the small intestine of day old chicks (Pedroso *et al.*, 2006) and are thought to be introduced either at the pre-hatching stage (in the incubator or hatcher) or at the post-hatching stage (during transit from the hatchery). Our results support the fact that the gut is rapidly colonised post-hatch, as bacterial communities were detectable at 3 days post-hatch and more importantly were shown to be influenced by in-feed antimicrobials. This narrow window of opportunity for influencing the gut colonisation could potentially be exploited by modifying mechanical process already in place in commercial hatcheries such as use of spray inoculation to administer a probiotic to newly hatch chicks or *in-ovo* inoculation to administer a prebiotic.

Chapter 2: Identification of performance related gut microbiota – Trial 1

Introduction

Previous research (CRC project 03-3a) demonstrated that overall changes in gut microbiota associated with non-starch polysaccharide (NSP) degrading enzyme supplementation were correlated with improved bird performance (Torok *et al*., 2008). However, it was not know whether this was a cause or effect of improved performance. The aim of this study was to determine if particular gut bacteria could be consistently linked with either improved or decreased performance across three Australian broiler performance trials. This chapter will describe investigations into the gut microbiota of broiler chickens in the first of three collaborative performance trials. Trial 1 was done at the Queensland Poultry Research Centre, Alexandra Hills, Queensland in May 2007. This trial (Evaluation of sorghum grains from Queensland and New South Wales for broiler growth performance in a semi-commercial environment) was led by Dr R Perez-Maldonado and supported by RIRDC (project DAQ-326A: Nutritional characteristics of sorghums from Queensland and New South Wales for chicken meat production).

Materials and methods

Trial 1

Trial description

Trial 1 investigated broiler performance on different dietary regimes with specific focus on sorghum varieties as detailed by Perez-Maldonado and Rodrigues (2009). Day old Arbor Acres broiler chicks (n=1800; 900 males and 900 females) were assigned to one of 60 floor pens (n=30/pen; 15 male and 15 female) at the Poultry Research Centre, Department of Primary Industries and Fisheries, Alexandra Hills, Queensland. Broilers received one of 10 dietary regimes (n=6 pens/treatment) with birds receiving starter diet from 0-21 days and then grower/finisher diet from 22-42 days. All animal experimentation was approved by the Department of Primary Industries and Fisheries Queensland Government Animal Ethics committee.

Diet composition

Gut microbiota was investigated from four out of a possible 10 grower/finisher dietary regimes being evaluated in the RIRDC trial. These four dietary regimes (Table 2.1) were expected to result in significant performance differences (R. Perez-Maldonado, personal communication).

Table 2.1: Composition of diets fed to broilers from which gut microbiota was investigated (RIRDC DAQ-326A 2007 floor pen growth trial)*.

Grower/Finisher Phase Ingredients (g/kg)	Wheat control plus xylanase	Sorghum B	Sorghum commercial	Sorghum commercial plus phytase
Wheat	657.81	0.00	0.00	0.00
Sorghum	0.00	639.10	654.78	654.78
Soybean meal	171.31	214.28	199.91	199.91
Canola meal	40.00	40.00	40.00	40.00
Meat/bone meal	40.00	40.00	40.00	40.00
Sunflower meal	30.00	30.00	30.00	30.00
Soybean oil	44.15	20.01	18.70	18.70
Limestone	1.71	1.34	1.40	1.40
Salt	1.94	1.25	1.24	1.24
Sodium bicarbonate	2.00	2.00	2.00	2.00
Krynofos[‡]	0.00	1.08	1.14	1.14
Coccidiostat[#]	0.50	0.50	0.50	0.50
Vitamins/Minerals	5.0	5.0	5.0	5.0
Lysine HCl	2.69	2.62	2.60	2.60
DL Methionine	2.38	2.59	2.73	2.73
Threonine	0.51	0.24	0.00	0.00
Enzyme xylanase	0.30	0.00	0.00	0.00
Enzyme phytase	0.00	0.00	0.00	0.15
Total	1000.30	1000.00	1000.00	1000.15
Calculated analysis (%)				
Crude protein	22.2	22.7	22.7	22.7
Lysine	1.10	1.10	1.10	1.10
Sulphur amino acids	0.84	0.84	0.84	0.84
Threonine	0.73	0.73	0.73	0.73
Isoleucine	0.75	0.80	0.83	0.83
Tryptophan	0.26	0.28	0.28	0.28
Arginine	1.26	1.26	1.23	1.23
Calcium	0.68	0.68	0.68	0.68
Available phosphorous	0.34	0.34	0.34	0.34
Calcium/available phosphorous	1.98	2.00	2.00	2.00
AME (MJ/kg)	13.0	13.0	13.0	13.0

* Information presented in the above table was taken from grower/finished diet composition (Table 27, Perez-Maldonado and Rodrigues, 2009).
[#] Type of coccidiostat was not able to be identified.
[‡]Krynofos contains monocalcium phosphate and dicalcium phosphate dihydrate in the ratio of approximately 3:1.

Bird performance

Performance was measured by live weight gain, feed intake and FRC. Data was statistically analysed using ANOVA and significant ($P<0.05$) differences between treatment means determined using the Least Significant Difference (LSD) test in GenStat™ (Perez-Maldonado and Rodrigues, 2009). Performance of broilers on all 10 dietary regimes is presented for the grower/finisher and overall periods (Table 2.2).

Microbial profiling

Samples collection and nucleic acid extraction

At 42 days of age two male birds per pen were taken from each of four dietary regimes: wheat control with xylanase (n=12); sorghum B (n=12); commercial sorghum (n=12) and commercial sorghum with added phytase (n=12). Birds were euthanized by cervical dislocation. Approximately a 2 cm section of the ileum (tissue and associated digesta), midway between the Meckel's diverticulum and caecal junction, as well as one caecum were collected from each chicken. Following collection samples were kept on ice until frozen at -20^{o}C and later freeze dried. Total nucleic acid was extracted from chicken gut as described in chapter 1.

T-RFLP and statistical analysis

T-RFLP and statistical analysis were done as described in chapter 1. Bray-Curtis measures of similarity (Bray and Curtis, 1957) were calculated to examine similarities between gut microbial communities of birds from the T-RFLP generated (OTU) data matrices, following standardization and fourth root transformation. One-way ANOSIM was used to test if gut microbial communities were significantly different between treatments. SIMPER (Clarke, 1993) analyses were done to determine which OTU contributed most to the dissimilarity between treatments. The overall average dissimilarity ($\bar{\delta}$) between gut microbial communities of birds on differing diets were calculated and the average contribution of the *i*th OTU ($\bar{\delta}_i$) to the overall dissimilarity determined. Average abundance ($\bar{y}$) of important OTU in each of the groups were determined. OTU contributing significantly to the dissimilarity between treatments were calculated ($\bar{\delta}_i/SD(\delta_i)>1$). Percent contribution of individual OTU ($\bar{\delta}_i\%$) and cumulative percent contribution ($\Sigma\ \bar{\delta}_i\%$) to the top 60% of average dissimilarities were also calculated.

Results

Bird performance

Gut samples were collected from birds fed four of the possible ten dietary regimes being investigated, without prior knowledge of performance results. However, selection of the four diets was based on previous observations of performance differences on these types of diets (R Perez-Maldonado, personal communication). Following completion of the trial it was shown that birds on wheat control diet with added xylanase had significantly increased feed intake compared to birds on the sorghum B diet at 22-42 days (Table 2.2; Perez-Maldonado and Rodrigues, 2009). Live weight gain at both 22-42 and 0-42 days was significantly decreased for birds on the wheat control diet with added xylanase compared to those on the sorghum B, sorghum commercial and sorghum commercial with added phytase diets (Table 2.2; Perez-Maldonado and Rodrigues, 2009). Feed conversion efficiency was significantly decreased for birds on the wheat control diet as compared to the three sorghum based diets at both 22-42 and 0-42 days (Table 2.2; Perez-Maldonado and Rodrigues, 2009).

Table 2.2: Broiler performance data for the 2007 floor pen growth trial*.

Treatment	Feed intake (g/bird)		Live weight gain (g/bird)		FCR (g/g)	
Age (days)	**22-42d**	**0-42d**	**22-42d**	**0-42d**	**22-42d**	**0-42d**
Wheat control + xylanase[#]	3567^{bc}	4637^{abc}	1803^{d}	2595^{d}	1.986^{e}	1.788^{d}
Sorghum H	3464^{a}	4549^{ab}	1920^{a}	2681^{ab}	1.810^{bd}	1.702^{b}
Sorghum M	3607^{b}	4721^{c}	1980^{c}	2777^{c}	1.855^{ac}	1.719^{ab}
Sorghum B[#]	3466^{a}	4582^{ab}	1894^{ab}	2679^{ab}	1.847^{abc}	1.721^{ab}
Sorghum E	3605^{b}	4715^{c}	1923^{a}	2720^{ac}	1.880^{a}	1.735^{ac}
Sorghum commercial[#]	3535^{ab}	4614^{abc}	1908^{ab}	2675^{ab}	1.854^{abc}	1.728^{abc}
Sorghum K	3473^{ac}	4526^{b}	1907^{ab}	2651^{bd}	1.831^{cd}	1.718^{ab}
Wheat (Starter)/sorghum (Finisher)	3489^{ac}	4573^{ab}	1857^{bd}	2657^{bd}	1.882^{a}	1.722^{ab}
Sorghum commercial + phytase[#]	3532^{ab}	4649^{ac}	1898^{ab}	2674^{ab}	1.863^{ac}	1.739^{ac}
Sorghum commercial low AvP + phytase	3517^{ab}	4645^{ac}	1884^{ab}	2666^{ab}	1.878^{a}	1.749^{c}
LSD (P=0.05)	94.8	117.4	56.5	62.2	0.041	0.026

Different superscripts in columns indicate significantly (P<0.05) different means.
* Information presented was condensed from Table 28, Perez-Maldonado and Rodrigues, 2009.
[#] Dietary treatments from which birds were taken for gut microbial investigation.

Gut microbial profiles

Diet had no influence on caecal microbial community composition (Global R=0.048, P=0.075), however, diet did have an influence on the ileal microbial community composition (Table 2.3). Ileal microbial communities were significantly different (P<0.05) between the wheat control diet and either of the sorghum commercial diets.

Similarity in ileal microbial community composition for birds on the same diet ranged from 32 to 48%, with birds on the wheat control diet having the lowest similarity and birds on the sorghum commercial diet with added phytase having the highest similarity. Similarity in caecal microbial communities for birds on the same diet were generally lower (24 to 37%) with similarity being the lowest for birds on the control wheat diet and highest for birds on the sorghum commercial diet.

OTU contributing to the top 60% of dissimilarity in ileal bacterial community composition between the wheat control and sorghum commercial diets (Table 2.4) and wheat control and sorghum commercial with added phytase diets (Table 2.5) were determined.

Table 2.3: One-way ANOSIM of ileal microbial communities associated with diet. The R statistic (**bold**) and significance level (*italics*) are shown between pair wise comparisons. Global R=0.079 and P=0.015.

	Wheat + xyl	Sorg B	Sorg comm	Sorg comm + phyt
Wheat + xyl*		**0.043**	**0.221**	**0.128**
Sorg B	*0.178*		**0.034**	**-0.019**
Sorg comm	*0.007*	*0.233*		**0.082**
Sorg comm + phyt	*0.042*	*0.562*	*0.089*	

*Wheat + xyl = wheat control diet with added xylanase; Sorg B = sorghum B diet; Sorg comm = sorghum commercial diet; Sorg comm + phyt = sorghum commercial with added phytase.

Table 2.4: OTU contributing to the dissimilarity in ileal microbial communities associated with diet. Average abundance of important OTU in ileal microbial communities of birds fed either the wheat control diet with added xylanase (wheat +xyl) or the sorghum commercial (Sorg comm) diets are shown. OTU are listed in order of their contribution ($\overline{\delta}_i$) to the average dissimilarity $\overline{\delta}$ (=71.43%) between dietary treatments. Percent contribution of individual OTU and cumulative percent contribution to the top 60% of average dissimilarities are shown. OTU contribution significantly to the dissimilarity between dietary treatments were calculated ($\overline{\delta}_i/SD(\delta_i)>1$) and are marked with and asterix.

OTU	Average abundance		$\overline{\delta}_i$	$\overline{\delta}_i/SD(\delta_i)$	Individual Contribution %	Cumulative Contribution %
	Wheat + xyl	Sorg comm				
180*	1.14	1.61	5.60	1.14	7.84	7.84
564*	1.75	1.02	5.42	1.33	7.59	15.42
492*	1.51	1.76	4.89	1.22	6.84	22.27
468*	0.00	1.01	3.95	1.08	5.52	27.79
454	0.90	0.13	3.49	0.81	4.89	32.68
566	0.33	0.75	3.44	0.90	4.82	37.50
600*	0.20	0.88	3.43	1.17	4.80	42.30
936*	0.58	0.98	3.34	1.19	4.68	46.98
178	0.58	0.28	2.68	0.77	3.76	50.73
188	0.00	0.70	2.58	0.82	3.61	54.34
76*	0.10	0.63	2.42	1.14	3.38	57.73
912	0.11	0.57	2.41	0.83	3.37	61.10

Table 2.5: OTU contributing to the dissimilarity in ileal microbial communities associated with diet. Average abundance of important OTU in ileal microbial communities of birds fed either the wheat control diet with added xylanase (wheat +xyl) or the sorghum commercial with added phytase (Sorg comm + phyt) diets are shown. OTU are listed in order of their contribution ($\overline{\delta}_i$) to the average dissimilarity $\overline{\delta}$ (=64.48%) between dietary treatments. Percent contribution of individual OTU and cumulative percent contribution to the top 60% of average dissimilarities are shown. OTU contribution significantly to the dissimilarity between dietary treatments were calculated ($\overline{\delta}_i/SD(\delta_i)>1$) and are marked with and asterix.

OTU	Average abundance		$\overline{\delta}_i$	$\overline{\delta}_i/SD(\delta_i)$	Individual Contribution %	Cumulative Contribution %
	Wheat + xyl	Sorg comm + phyt				
180*	1.14	1.62	5.42	1.23	8.41	8.41
454	0.90	1.04	5.24	0.99	8.12	16.53
468*	0.00	1.23	4.78	1.22	7.42	23.95
564*	1.75	1.58	4.49	1.32	6.96	30.91
492*	1.51	1.69	3.35	1.20	5.19	36.11
936*	0.58	1.03	3.19	1.19	4.95	41.05
76*	0.10	0.82	3.15	1.53	4.89	45.95
504	0.33	0.69	2.96	0.97	4.59	50.54
178	0.58	0.43	2.95	0.83	4.57	55.11
566	0.33	0.51	2.79	0.71	4.32	59.43
888	0.00	0.63	2.42	0.96	3.76	63.19

Discussion

In trial 1 broiler chickens at 42 days of age performed best (as measured by FCR and body weight gain) on the three sorghum based diets as compared to the control wheat based diet. Ileal microbial community composition was also significant different between birds fed the wheat control diet as compared to either of the sorghum commercial diets. Six OTU (76, 180, 468, 564, 492 and 936) were identified as contributing significantly to the difference between these dietary treatments. OTU 76, 180, 468, 492 and 936 were more abundant in the better performing sorghum commercial diets, while OTU 564 was more abundant in the poorer performing wheat control diet.

No significant differences were detected in the caecal microbial communities between birds fed the four diets investigated, although there was a tendency towards significance (P=0.075). Inter-bird variation in gut microbial communities has previously been reported to vary for chickens on the same diet (Torok *et al*., 2008; Torok *et al*., 2009). When using T-RFLP to investigate broiler gut microbiota the similarity in bacterial communities for birds on the same treatment have been reported in the range of 25 to 48%. This level of similarity (24 to 48%) was observed for birds on the same dietary treatment in this current trial; however, the caecal microbial communities tended to have a much lower similarity (24 to 37%). This higher inter-bird variation, in combination with the low number of replicate birds per treatment (n=12) may have resulted in the absence of detectable differences in caecal microbial communities associated with diet. For this reason gut microbial profiling done on all subsequent performance trial used n=24 birds/treatment.

Chapter 3: Identification of performance related gut microbiota – Trial 2

Introduction

We wished to determine if particular gut bacteria could be consistently linked with improved or decreased broiler performance across three Australian performance trials. This chapter presents data from the second of the three collaborative performance trials. Trial 2 was done at Inghams Enterprise Research Facility, Leppington, New South Wales in March 2008. This trial (The effect of litter and dietary fibre on gut development, nutrient digestibility and gut microbiota) was led by Dr L Mikkelsen, University of New England and supported by the Australian Poultry CRC (project 06-18: Role of voluntary litter consumption by broiler chickens on gut function and gut health). The microbiota from the ilea and caeca of broiler chickens were investigated using a bacterial profiling technique to determine if two types of litter (paper and wood) in combination with a low or high fibre diet affected gut microbial communities and could be linked to performance.

Materials and methods

Trial 2

Trial description

720 day-old Cobb 500 broiler chickens were raised for 6 weeks in 24 floor pens (n=30 birds/pen) in a temperature-controlled shed at Inghams Enterprise Research Facility in Leppington, New South Wales. Each pen was randomly assigned to one of four treatment groups (n=6 pens/treatment) in a 2 x 2 factorial design arrangement. Half of the replicate pens within a treatment contained all male birds (n=90) while the other half contained all female birds (n=90).

Diet composition

Chickens in the experiment were raised from hatch until 42 days of age on one of four treatments. Birds were raised on one of two litter materials (paper or hardwood) in combination with either a low or high fibre diet (Table 3.1). At the time gut microbial communities were investigated (day 35) birds were moving from finisher to withdrawal diet (34 to 36 days of age).

Bird performance

Bird performance, as measured by body weight and FCR, was determined at 7, 14, 35 and 42 days of age. Data were statistically analysed by Dr L Mikkelsen, University of New England. As sex was found to be a significant factor (L. Mikkelsen, personal communication), performance data for males and females were further analysed separately and presented in table 3.2 and 3.3 respectively.

Table 3.1: Experimental diets

Nutrients	Energy (MJ/Kg)	Protein (%)	Fat (%)	Fibre (%)
Starter*#				
Control	12.8	23.5	6.2	3.0
High fibre	12.1	22.2	6.2	5.0
Grower*#				
Control	12.9	21.0	6.2	3.0
High fibre	12.2	20.0	6.2	5.0
Finisher*#				
Control	13.1	20.0	6.2	3.0
High fibre	12.4	19.0	6.2	5.0
Withdrawal#				
Control	13.1	19.4	6.2	3.0
High fibre	12.4	18.4	6.3	5.0

Ingredients	Control (%)	High fibre (%)
Wheat	64.6	59.4
Soymeal	18.1	16.4
Meat meal	6.6	6.6
Expellor canola meal	5.0	5.0
Oat hulls	-	7.0
Poultry tallow	3.8	3.8
Vitamins & minerals	1.9	1.8

* Diets contained salinomycin (60 ppm), dinitolamide (125 ppm) and flavophospholipol (2 ppm)
# Diets contained zinc bacitracin (50 ppm)

Table 3.2: Growth performance data for male broilers

	Paper		Hard-wood		SEM	Litter	Fibre	Litter x Fibre
	High Fibre	Low Fibre	High Fibre	Low Fibre				
Mean body weight (g)								
Day 7	185	175	179	180	3	NS	NS	NS
Day 21	984^{c}	916^{a}	955^{b}	952^{b}	7.7	NS	**	**
Day 35	2324	2255	2285	2275	28.5	NS	NS	NS
Day 42	3017	2901	2897	2886	28.7	NS	NS	NS
Age at 2.45kg (days)	34.5	36.1	36.1	36.3	0.4	NS	NS	NS
FCR (feed/gain)								
Day 7	0.74^{a}	0.88^{b}	0.82^{ab}	0.78^{ab}	0.03	NS	NS	*
Day 21	1.18^{ab}	1.22^{b}	1.21^{b}	1.15^{a}	0.01	NS	NS	**
Day 35	1.49	1.50	1.49	1.45	0.02	NS	NS	NS
Day 42	1.65	1.64	1.67	1.63	0.03	NS	NS	NS

Values within a row not having the same letter are significantly different.
NS is P>0.05; * is 0.05>P>0.01; ** is 0.01>P>0.001

Table 3.3: Growth performance data for female broilers

	Paper		Hard-wood		SEM	Litter	Fibre	Litter x Fibre
	High Fibre	Low Fibre	High Fibre	Low Fibre				
Mean body weight (g)								
Day 7	181	174	175	173	2.3	NS	NS	NS
Day 21	869	862	881	881	6.6	*	NS	NS
Day 35	1987	1933	2021	2032	14.3	**	NS	NS
Day 42	2591^{b}	2453^{a}	2535^{ab}	2553^{b}	29.3	NS	NS	*
Age at 2.45kg (days)	40.2^{a}	42.0^{b}	40.9^{ab}	40.6^{a}	0.4	NS	NS	*
FCR (feed/gain)								
Day 7	0.81^{ab}	0.84^{b}	0.82^{ab}	0.79^{a}	0.01	NS	NS	*
Day 21	1.24^{bc}	1.22^{b}	1.26^{c}	1.19^{a}	0.01	NS	***	**
Day 35	1.55^{ab}	1.58^{b}	1.56^{b}	1.51^{a}	0.02	NS	NS	*
Day 42	1.67	1.75	1.73	1.70	0.03	NS	NS	NS

Values within a row not having the same letter are significantly different.
NS is P>0.05; * is 0.05>P>0.01; ** is 0.01>P>0.001; *** is P<0.001

Microbial profiling

Sample collection and nucleic acid extraction

At 35 days of age four birds per pen were taken from each of the four treatments: paper litter and low fibre diet; wood litter and low fibre diet; paper litter and high fibre diet; and wood litter and high fibre diet (n=24 birds/treatment; 12 males and 12 females). Birds were euthanized by cervical dislocation. Approximately a 2 cm section of the ileum (tissue and associated digesta), midway between the Meckel's diverticulum and caecal junction, as well as, one caecum were collected from each chicken. Following collection samples were kept on ice until frozen at -20^{o}C and later freeze dried. Total nucleic acid was extracted from chicken gut as described in chapter 1.

T-RFLP and statistical analysis

T-RFLP and statistical analysis were done as described in chapter 1 and 2. Bray-Curtis measures of similarity were calculated to examine similarities between gut microbial communities of birds from the T-RFLP generated (OTU) data matrices, following standardization and fourth root transformation. One-way ANOSIM was used to test if gut microbial communities were significantly different between treatments. SIMPER analyses were done to determine which OTU contributed most to the dissimilarity between treatments.

Results

Bird performance

Bird sex was found to significantly influence performance data (L. Mikkelsen, personal communication). Therefore, male and female bird data were further analysed separately. Litter treatment in combination with dietary fibre did not significantly influence body weight of either male (Table 3.2) or female (Table 3.3) birds at 35 days of age. However, at 21 days of age male birds fed the high fibre diet and raised on paper litter were significantly the heaviest while birds fed the low fibre diet and raised on paper litter were significantly the lightest (Table 3.2). Likewise at 42 days of age female birds fed the high fibre diet and raised on paper litter were the heaviest but only significantly different to those fed the low fibre diet and raised on paper litter (Table 3.3). Diet/litter combination did significantly influence FCR in female birds at 35 days of age. At 35 days of age male birds showed no significant differences in feed efficiency, although at 21 days of age they showed the

same significant differences as observed in the female group at 35 days of age. Significant differences were observed in FCR between female birds (day 35) and male birds (day 21) fed the low fibre diet and raised on hardwood shavings and birds fed either a low fibre diet and raised on paper litter or birds fed a high fibre diet and raised on hardwood litter.

Gut microbial profiles

Multivariate statistical analysis was used to investigate differences in gut microbial communities from either the ilea or caeca of birds. Factors investigated were litter/dietary fibre composition and sex of birds. No significant differences were detected in the ileal microbial community composition among litter/diet combinations (global R=0.025, P=0.110) across both sexes or between sexes of birds (global R=0.033, P=0.095) across all litter/diet treatments. However, significant differences were detected in the caecal microbial community composition among litter/diet combinations (global R=0.089, P=0.001) across both sexes and between sexes of birds (global R=0.046, P=0.034) across all litter/diet treatments. Therefore, caecal microbial communities from male (n=12/treatment) and female (n=12/treatment) birds were further analysed separately. Significant differences (P<0.05) were detected between: birds raised on paper and fed a low fibre diet versus birds raised on wood and fed either a low or high fibre diet; and birds raised on paper and fed a high fibre diet versus birds raised on wood and fed a high fibre diet for both males (Table 3.4) and females (Table 3.5).

Table 3.4: One-way ANOSIM of caecal microbial communities associated with litter/diet for male birds. The *R* statistic (**bold**) and significance level (*italics*) are shown between litter/diet treatments. Global R=0.084 and P=0.006.

	Paper + low fibre	Paper + high fibre	Wood + low fibre	Wood + high fibre
Paper + low fibre		**0.009**	**0.199**	**0.223**
Paper + high fibre	*0.371*		**0.031**	**0.116**
Wood + low fibre	*0.001*	*0.236*		**-0.048**
Wood + high fibre	*0.002*	*0.023*	*0.844*	

Table 3.5: One-way ANOSIM of caecal microbial communities associated with litter/diet for female birds. The *R* statistic (**bold**) and significance level (*italics*) are shown between litter/diet treatments. Global R=0.094 and P=0.001.

	Paper + low fibre	Paper + high fibre	Wood + low fibre	Wood + high fibre
Paper + low fibre		**0.025**	**0.128**	**0.172**
Paper + high fibre	*0.261*		**0.064**	**0.152**
Wood + low fibre	*0.018*	*0.080*		**0.047**
Wood + high fibre	*0.002*	*0.005*	*0.167*	

Multivariate statistical analysis showed that the composition of the caecal microbial community for both sexes was significantly different between litter materials but not between dietary treatments (Table 3.6).

Table 3.6: Two-way crossed ANOSIM of caecal microbial communities associated with sex for litter material and dietary fibre level. The global R statistic (**bold**) and significance level (*italics*) are shown for each of the factors for males and females separately.

	Litter	Diet
Female	**0.140**, *0.002*	**0.036**, *0.157*
Male	**0.157**, *0.001*	**-0.019**, *0.692*

OTU contributing to the top 60% of dissimilarity in caecal bacterial community composition between birds fed a low fibre diet and raised on either paper or hardwood litter were identified for male (Table 3.7) and female (Table 3.8) birds separately. Twenty-two OTU for males and twenty-two OTU for females were identified as being good discriminators between litter materials for birds fed the low fibre diet. Seventeen of these litter specific OTU were common to both sexes and included OTU 92/94, 142, 198/200, 206/208, 216/218, 222, 284/286, 300, 312, 482, 542 and 522.

Table 3.7: OTU contributing to the dissimilarity in caecal microbial communities from males fed a low fibre diet and raised on either paper or wood litter material. Average abundance of important OTU in caecal microbial communities of males are shown and listed in order of their contribution ($\overline{\delta}_i$) to the average dissimilarity $\overline{\delta}$ (=53.09%) between treatments. Percent contribution of individual OTU and cumulative percent contribution to the top 60% of average dissimilarities are shown. OTU contributing significantly to the dissimilarity between litter treatments were calculated ($\overline{\delta}_i/SD(\delta_i)>1$) and are marked with an asterix.

OTU	Average Abundance		$\overline{\delta}_i$	$\overline{\delta}_i/SD(\delta_i)$	Individual Contribution %	Cumulative contribution %
	Low Fibre Diet					
	Paper litter	Wood litter				
284*	1.62	0.46	2.01	1.65	3.79	3.79
92*	1.01	1.97	1.63	1.26	3.06	6.86
94*	0.77	1.53	1.58	1.28	2.98	9.84
542*	0.46	1.31	1.49	1.57	2.81	12.65
286*	1.15	1.05	1.37	1.14	2.58	15.23
218*	0.95	0.46	1.34	1.20	2.53	17.76
522*	0.95	0.43	1.34	1.20	2.52	20.27
300*	0.63	1.17	1.28	1.23	2.40	22.68
206*	0.39	1.01	1.21	1.30	2.28	24.96
222*	0.59	0.87	1.18	1.17	2.22	27.18
288*	0.64	0.42	1.15	1.05	2.17	29.35
116*	0.66	0.72	1.11	1.14	2.10	31.45
482*	0.89	0.51	1.09	1.20	2.05	33.50
536*	0.64	0.77	1.05	1.16	1.99	35.48
280*	0.82	0.75	1.04	1.10	1.96	37.44
190	0.53	0.46	1.04	0.99	1.95	39.39
302	0.41	0.52	1.03	0.94	1.95	41.34
198*	0.55	0.56	1.03	1.11	1.94	43.28
292*	0.64	0.48	1.03	1.08	1.93	45.22
208*	0.28	0.68	1.02	1.11	1.93	47.15
140	1.08	1.25	0.98	0.99	1.84	48.98
142*	1.24	1.29	0.97	1.08	1.82	50.81
312*	1.13	0.93	0.96	1.02	1.81	52.61
90*	0.37	0.65	0.95	1.07	1.79	54.40
296*	1.12	1.49	0.91	1.04	1.72	56.12
144	0.39	0.49	0.90	0.99	1.70	57.82
282	0.42	0.42	0.89	0.91	1.67	59.49

Table 3.8: OTU contributing to the dissimilarity in caecal microbial communities from females fed a low fibre diet and raised on either paper or wood litter material. Average abundance of important OTU in caecal microbial communities of females are shown and listed in order of their contribution ($\bar{\delta}_i$) to the average dissimilarity $\bar{\delta}$ (=56.55%) between treatments. Percent contribution of individual OTU and cumulative percent contribution to the top 60% of average dissimilarities are shown. OTU contributing significantly to the dissimilarity between litter treatments were calculated ($\bar{\delta}_i/SD(\delta_i)>1$) and are marked with an asterix.

OTU	Average Abundance		$\bar{\delta}_i$	$\bar{\delta}_i/SD(\delta_i)$	Individual contribution %	Cumulative contibution %
	Low Fibre Diet					
	Paper litter	Wood litter				
286*	1.23	0.81	1.47	1.18	2.61	2.61
218*	0.90	0.63	1.39	1.17	2.46	5.06
142*	0.93	1.05	1.37	1.14	2.43	7.49
542*	0.47	1.31	1.34	1.45	2.37	9.86
288	0.63	0.56	1.29	0.98	2.27	12.14
312*	1.26	0.72	1.28	1.19	2.27	14.41
284*	0.89	0.59	1.28	1.23	2.26	16.67
94*	0.90	1.51	1.26	1.19	2.23	18.90
92*	1.16	1.90	1.26	1.13	2.23	21.13
222*	0.85	0.69	1.24	1.14	2.19	23.32
180*	0.27	0.74	1.21	1.13	2.14	25.46
566*	0.36	0.80	1.20	1.29	2.13	27.58
300*	0.95	0.72	1.16	1.18	2.05	29.63
216*	0.73	0.77	1.15	1.15	2.03	31.66
522*	0.45	0.68	1.12	1.04	1.98	33.64
144*	0.78	0.40	1.08	1.20	1.91	35.55
206*	0.47	0.97	1.08	1.21	1.90	37.45
84*	1.15	0.62	1.05	1.08	1.86	39.31
282*	0.61	0.43	1.03	1.05	1.83	41.14
482*	0.43	0.64	1.03	1.03	1.82	42.96
198*	0.41	0.68	1.02	1.12	1.81	44.77
492	0.22	0.56	1.01	0.78	1.78	46.56
208*	0.17	0.65	0.99	1.12	1.76	48.31
200*	0.58	0.86	0.99	1.12	1.75	50.07
476	0.45	0.45	0.94	0.97	1.66	51.73
140	1.06	1.12	0.88	0.99	1.56	53.29
182	0.25	0.44	0.88	0.70	1.56	54.85
536	0.50	0.37	0.88	0.97	1.55	56.40
188	0.43	0.26	0.87	0.82	1.54	57.94
292	0.32	0.41	0.87	0.85	1.54	59.48

Discussion

Microbial profiling of the ileal samples from trial 2 showed there were no significant ($P>0.05$) differences between any of the dietary/litter treatments. However, caecal microbial communities were significantly different between sexes and diets. Caecal microbial communities for both male and female birds varied across litter materials but not dietary treatments. In both male and female broilers fed a low fibre diet the caecal microbial communities were significantly ($P<0.05$) different between birds reared on paper versus hardwood litter. Significant performance differences as measured by FCR were also detected at 35 days of age in female birds on these two treatments, with improved feed efficiency in birds fed a low fibre diet and raised on hardwood litter. OTU 84, 92/94, 142/144, 180,

198/200, 206/208, 216/218, 222, 282, 284/286, 300, 312, 482, 542, 522 and 566 were identified as contributing to differences in caecal microbial communities between female birds on the low fibre diet/paper litter and low fibre diet/hardwood litter. OTU 92/94, 142, 180, 198/200, 206/208, 216, 482, 542, 522 and 566 were more abundant in the group with improved performance while OTU 84, 144, 218, 222, 282, 284/286, 300 and 312 were more abundant in the lower performing group. Seventeen of these discriminating OTU (92/94, 142, 198/200, 206/208, 216/218, 222, 284/286, 300, 312, 482, 542 and 522) were also found to be in common with male broilers fed the low fibre diet and raised on either paper or hardwood litter, despite no differences being detected in their performance at 35 days of age. It is difficult to say if the potential performance related OTU identified in females are truly indicators of performance or merely a result of treatment, as the males also showed similar caecal microbial differences. However, it should be noted that the male broilers did exhibit similar significant performance differences between the exact same treatments as observed in the female birds (35 days) but at a much younger age (21 days).

Chapter 4: Identification of performance related gut microbiota – Trial 3

Introduction

In order to determine if specific gut bacteria could be consistently linked with improved or decreased broiler performance we investigated gut microbial communities from three Australian performance trials. This chapter presents data from the third of the three collaborative performance trials. Trial 3 was done at Inghams Enterprise Research Facility, Leppington, New South Wales in November 2008. Trial 3 was led by Dr R MacAlpine and K Balding and was evaluating commercial broiler feeds produced in various feed mills across Australia. The microbiota from the ilea and caeca of broiler chickens were investigated using a bacterial profiling technique to determine if feed type affected gut microbial communities and could be linked to performance.

Materials and methods

Trial 3

Trial description

960 day-old Cobb 500 broiler chickens were raised for 6 weeks in 32 floor pens in a temperature-controlled shed at Inghams Enterprise Research Facility in Leppington, New South Wales. Each pen (n=30 birds/pen) was randomly assigned to one of eight treatments in a 2 x 2 factorial design with four replicate pens per treatment (n=2 male and n=2 female).

Diet composition

The composition of the four withdrawal diets on which broilers were being fed at the time gut microbial communities were investigated (42 days of age) are shown in Table 4.1.

Bird performance

Performance data, as measured by corrected FCR at 2.6 kg, were analysed with SAS for Windows version 9.1 software package (Base SAS software; SAS Institute Inc., Cary, NC, USA) using the General Linear Model (GLM) with differences between treatments determined by Duncan's Multiple Range Test.

Microbial profiling

Sample collection and nucleic acid extraction

At 42 days of age six birds per pen were taken from each of four dietary treatments A, B, F and G; n=24 birds/treatment. Birds were euthanized by cervical dislocation. Approximately a 2 cm section of the ileum (tissue and associated digesta), midway between the Meckel's diverticulum and caecal junction, as well as one caecum were collected from each chicken. Following collection samples were kept on ice until frozen at -20^{o}C and later freeze dried. Total nucleic acid was extracted from chicken gut as described in chapter 1.

Table 4.1: Composition of diets

	WITHDRAWAL DIET (%)			
Raw material	**Diet A**	**Diet B**	**Diet F**	**Diet G**
Wheat	15.955	15.057	50.021	39.985
Sorghum	60.360	57.695	-	33.227
Barley	-	-	15.143	-
Oats	-	-	7.143	-
Meat meal	4.800	4.250	4.714	6.500
Poultry meal	-	-	-	7.500
Soybean meal	15.500	10.750	7.714	11.000
Canola meal	-	7.000	6.000	-
Sunflower meal	-	2.000	-	-
Lupin meal	-	-	5.000	-
Limestone	0.700	0.525	0.571	0.600
Tallow/Oil	1.400	1.500	2.571	-
Lysine	0.302	0.320	0.300	0.385
Methionine hydroxy analogue	0.210	0.178	0.189	0.200
Sodium bicarbonate	0.260	0.275	0.286	0.250
Salt	0.140	0.150	0.114	0.050
Xylanase	-	0.015	0.025	0.030
Phytase	0.015			-
Coccidiostat*	-	-	-	0.093
Surmax[#]	0.015	0.015	0.015	0.015
Zinc bacitracin	-	0.070	-	-
Premix	0.343	0.200	0.194	0.165
TOTAL	100.000	100.000	100.000	100.000

*Coccidiostat used was Elancoban (Elanco) which contains monensin sodium.
[#] Surmax (Elanco) contains avilamycin

T-RFLP and statistical analysis

T-RFLP and statistical analysis were done as described in chapter 1 and 3. Bray-Curtis measures of similarity were calculated to examine similarities between gut microbial communities of birds from the T-RFLP generated (OTU) data matrices, following standardization and fourth root transformation. One-way ANOSIM was used to test if gut microbial communities were significantly different between diets. SIMPER analyses were done to determine which OTU contributed most to the dissimilarity between treatments. Unconstrained ordinations were done to graphically illustrate the relationships between diet and performance level by using nonmetric multidimensional scaling (nMDS) (Kruskal, 1964; Shepard, 1962). nMDS ordinations attempt to place all samples in an arbitrary two-dimensional space such that their relative distances apart match the corresponding pair wise similarities. Hence, the closer two samples are in the ordination the more similar are their overall gut bacterial communities. “Stress” values (Kruskal’s formula 1) reflect the difficulty involved in compressing the sample relationship into the two dimensional ordination.

Results

Bird performance

Birds fed diets A and B had significantly reduced feed efficiencies as compared with birds fed diets F and G (Table 4.2). Birds fed diet G had the most significantly improved feed efficiency of birds on any of the investigated diets.

Table 4.2: Broiler performance data

Diet	Corrected FCR at 2.6 kg
A*	1.58 ± 0.05^{ab}
B*	1.60 ± 0.06^{a}
C	1.56 ± 0.06^{bc}
D	1.57 ± 0.07^{bc}
E	1.59 ± 0.05^{ab}
F*	1.55 ± 0.07^{c}
G*	1.50 ± 0.05^{d}
H	1.56 ± 0.07^{bc}
Two-way ANOVA	
Diet	<0.0001
Sex	<0.0001
Diet x Sex	0.0243

* Indicates diets fed to broilers for which gut microbial communities were investigated.

Gut microbial profiles

Multivariate statistical analysis was used to investigate differences in gut microbial communities from either the ilea or caeca of birds. Factors investigated were diet and sex of birds. No significant differences were detected in the ileal microbial community composition between sexes of birds (global R=0.031, P=0.127), however significant differences were detected among diets (global R=0.331, P=0.001) across both sexes (Table 4.3).

Table 4.3: Two-way ANOSIM of ilea microbial communities associated with sex and diet. The *R* statistic (**bold**) and significance level (*italics*) are shown for each dietary pair wise comparisons. Diet (Global R=0.331, P=0.001) and sex (Global R=0.031, P=0.127).

	Diet A	Diet B	Diet F	Diet G
Diet A		**0.003**	**0.202**	**0.579**
Diet B	*0.418*		**0.231**	**0.515**
Diet F	*0.001*	*0.001*		**0.472**
Diet G	*0.001*	*0.001*	*0.001*	

Significant differences (global R=0.253, P=0.001) were also observed in ilea microbial communities between improved performing birds (fed diets F and G) and poorer performing birds (fed diets A and B). OTU were identified which contributed to the top 50% of dissimilarity in ileal bacterial community composition between improved and poorer performing birds (Table 4.4). Seven OTU (180, 188, 454, 492, 506, 566 and 938) were identified within the ilea as discriminating between poor and improved performance.

Table 4.4: OTU contributing to the dissimilarity in ileal bacterial communities of birds fed diets showing improved (diets F and G) or poorer (diets A and B) performance as measured by FCR. Average abundance of important OTU in ileal bacterial communities of birds showing either improved or poorer performance were identified. OTU are listed in order of their contribution ($\bar{\delta}i$) to the average dissimilarity $\bar{\delta}$ (=65.49%) between performance levels. Percent contribution of individual OTU and cumulative percent contribution to the top 50% of average dissimilarities are shown. OTU contributing significantly to the dissimilarity between performance levels were calculated ($\bar{\delta}_i/SD(\delta_i)>1$) and are marked with an asterix.

OTU	Average abundance		$\bar{\delta}i$	$\bar{\delta}_i/SD(\delta_i)$	Individual Contribution %	Cumulative Contribution %
	Poorer performance	Improved performance				
454*	1.25	1.46	4.98	1.18	7.61	7.61
180*	2.06	1.22	4.56	1.21	6.96	14.57
566*	2.07	1.10	4.50	1.26	6.87	21.43
188*	1.27	0.40	4.12	1.28	6.29	27.72
492*	0.70	1.21	4.08	1.17	6.23	33.96
938*	1.01	0.50	2.86	1.31	4.37	38.33
450	0.03	0.79	2.76	0.71	4.22	42.54
506*	0.65	0.55	2.61	1.04	3.98	46.53
576	0.63	0.03	2.25	0.92	3.44	49.97

Within the caeca, significant differences in microbial community composition were detected between the sexes across all diets (global R=0.281, P=0.001) and among diet across both sexes (global R=0.541, P=0.001; Table 4.5). Therefore, caecal microbial communities from male and female birds were separately analysed. Significant differences were also observed in caeca microbial communities between improved performing birds (diets F and G) and poorer performing birds (diets A and B) for both males (global R=0.535, P=0.001) and females (global R=0.297, P=0.001). Differences in caecal microbial communities of male birds fed the four diets are shown in Figure 4.1a, with separation into improved and poorer performing groups shown in Figure 4.1b.

Table 4.5: Two-way ANOSIM of caecal microbial communities associated with sex and diet. The R statistic (**bold**) and significance level (*italics*) are shown for each dietary pair wise comparison. Diet (global R=0.541, P=0.001) and sex (global R=0.281, P=0.001).

	Diet A	Diet B	Diet F	Diet G
Diet A		**0.473**	**0.741**	**0.499**
Diet B	*0.001*		**0.721**	**0.479**
Diet F	*0.001*	*0.001*		**0.394**
Diet G	*0.001*	*0.001*	*0.001*	

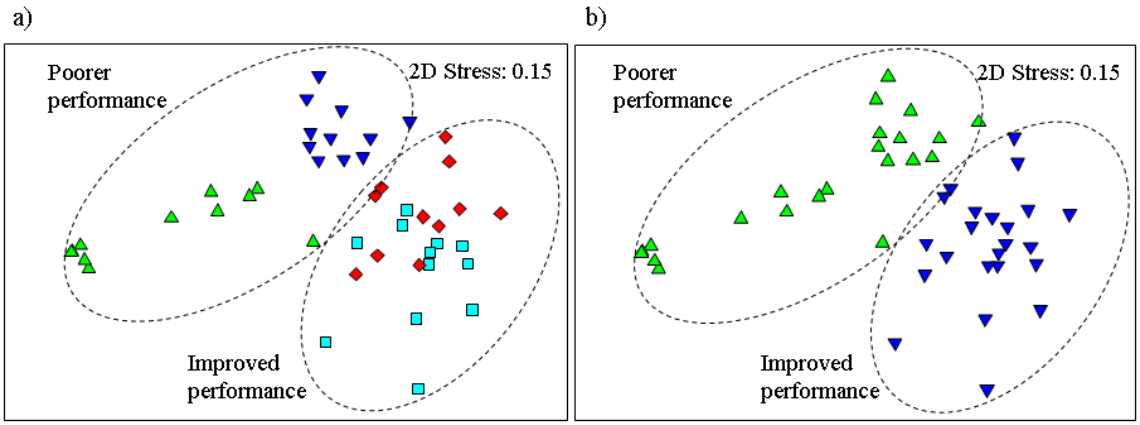

Figure 4.1: nMDS ordination of caecal microbial communities from males. (a) Caecal microbial communities identified by diet: ▲ diet A; ▼ diet B; ■ diet F; and ◆ diet G. (b) Same ordination as in (a), however microbial communities are identified as either being from the improved (▼) or poorer (▲) performing birds. The ordination is based on Bray-Curtis similarities calculated from standardized and 4th-root transformed OTU abundances. nMDS ordinations attempt to place all samples in an arbitrary two-dimensional space such that their relative distances apart match the corresponding pair-wise similarities. Hence, the closer two samples are in the ordination the more similar are their overall gut bacterial communities. "Stress" values (Kruskal's formula 1) reflect difficulty involved in compressing the sample relationship into the 2-D ordination.

OTU contributing to the top 50% of dissimilarity in caecal bacterial community composition between improved and poorer performing birds were identified for females (Table 4.6) and males (Table 4.7). Eleven discriminating OTU were identified in females (140, 142, 212, 218, 220, 282, 286, 312, 484, 488 and 536) and 16 discriminating OTU were identified in males (140, 142, 212, 218, 220, 284, 286, 312, 476, 482, 484, 488, 492, 528, 536 and 906).

Table 4.6: OTU contributing to the dissimilarity in caecal bacterial communities of females fed diets showing improved (diets F and G) or poorer (diets A and B) performance as measured by FCR. Average abundance of important OTU in caecal bacterial communities of birds with either improved or poorer performance were identified. OTU are listed in order of their contribution ($\bar{\delta}i$) to the average dissimilarity $\bar{\delta}$ (=73.46%) between performance levels. Percent contribution of individual OTU and cumulative percent contribution to the top 50% of average dissimilarities are shown. OTU contributing significantly to the dissimilarity between performance levels were calculated ($\bar{\delta}_i/SD(\delta_i)>1$) and are marked with an asterix.

OTU	Average abundance		$\bar{\delta}i$	$\bar{\delta}_i/SD(\delta_i)$	Individual Contribution %	Cumulative Contribution %
	Poorer performance	Improved performance				
94	1.00	0.31	2.04	0.83	2.78	2.78
284*	0.14	1.12	1.79	1.43	2.43	5.21
220*	0.84	1.50	1.77	1.38	2.41	7.62
482*	0.41	1.22	1.67	1.49	2.27	9.89
218*	1.11	1.16	1.54	1.18	2.09	11.99
286*	0.99	0.91	1.50	1.22	2.05	14.03
96	0.70	0.00	1.35	0.73	1.84	15.87
140*	0.51	0.95	1.29	1.17	1.75	17.63
212*	0.70	0.18	1.28	1.04	1.75	19.38
296	1.04	1.42	1.26	0.95	1.71	21.09
142*	0.45	0.78	1.24	1.10	1.68	22.77
294	0.71	0.16	1.23	0.97	1.67	24.45
492	0.00	0.69	1.22	0.75	1.66	26.11
474	0.27	0.60	1.19	0.76	1.63	27.74
546	0.55	0.19	1.17	0.76	1.60	29.33
312*	0.98	1.02	1.17	1.00	1.59	30.93
214	0.59	0.22	1.14	0.91	1.55	32.48
288	0.62	0.29	1.13	0.96	1.53	34.01
280	0.18	0.63	1.12	0.89	1.53	35.54
536*	0.72	0.75	1.12	1.07	1.53	37.07
282	0.09	0.65	1.11	0.90	1.52	38.59
476	0.68	0.15	1.10	0.97	1.50	40.09
144	0.16	0.60	1.08	0.93	1.48	41.56
488*	0.24	0.65	1.08	1.01	1.47	43.03
590	0.67	0.04	1.08	0.95	1.47	44.50
378	0.00	0.60	1.05	0.75	1.43	45.93
90	0.38	0.56	1.03	0.98	1.41	47.34
566	0.10	0.56	0.98	0.91	1.34	48.68
190	0.51	0.20	0.95	0.79	1.29	49.97

Table 4.7: OTU contributing to the dissimilarity in caecal bacterial communities of males fed diets showing improved (diets F and G) or poorer (diets A and B) performance as measured by FCR. Average abundance of important OTU in caecal bacterial communities of birds with either improved or poorer performance were identified. OTU are listed in order of their contribution ($\bar{\delta}i$) to the average dissimilarity $\bar{\delta}$ (=76.25%) between performance levels. Percent contribution of individual OTU and cumulative percent contribution to the top 50% of average dissimilarities are shown. OTU contributing significantly to the dissimilarity between performance levels were calculated ($\bar{\delta}_i/SD(\delta_i)>1$) and are marked with an asterix.

OTU	Average abundance		$\bar{\delta}i$	$\bar{\delta}_i/SD(\delta_i)$	Individual Contribution %	Cumulative Contribution %
	Poorer performance	Improved performance				
94	1.20	0.00	2.40	0.90	3.15	3.15
492*	0.08	1.17	2.08	1.30	2.73	5.88
220*	0.62	1.60	2.04	1.39	2.67	8.55
482*	0.17	1.21	2.00	1.59	2.62	11.17
96	0.93	0.00	1.85	0.93	2.42	13.60
140*	0.11	0.99	1.72	1.39	2.26	15.86
286*	0.93	1.03	1.64	1.17	2.15	18.01
476*	1.00	0.25	1.58	1.33	2.07	20.08
284*	0.12	0.88	1.52	1.13	2.00	22.08
546	0.74	0.00	1.48	0.87	1.94	24.01
142*	0.16	0.86	1.47	1.21	1.93	25.94
212*	0.75	0.00	1.47	1.02	1.92	27.87
488*	0.05	0.83	1.46	1.17	1.91	29.78
296	1.24	1.60	1.43	0.94	1.87	31.65
218*	0.60	0.61	1.38	1.00	1.80	33.46
312*	0.92	1.28	1.36	1.03	1.78	35.24
528*	0.86	0.35	1.35	1.19	1.77	37.01
214	0.60	0.09	1.26	0.80	1.66	38.67
536*	0.95	1.03	1.25	1.01	1.63	40.30
484*	0.74	0.10	1.24	1.05	1.63	41.93
288	0.61	0.31	1.23	0.89	1.62	43.55
522	0.00	0.66	1.21	0.99	1.58	45.13
144	0.21	0.63	1.15	0.97	1.51	46.64
906*	0.40	0.63	1.14	1.10	1.50	48.17
90	0.13	0.60	1.14	0.89	1.49	49.63

Discussion

In trial 3, feed conversion efficiency was significantly better for broilers fed diets F and G as compared with diets A and B. Both the poorer performing diets (A and B) contained a higher percentage of sorghum (57-60%) and only 15% wheat, whereas the improved performing diets had a higher percentage of wheat (40-50%) and either no sorghum (diet F) or a low percentage composition of sorghum (diet G; 33%).

Microbial profiling of the ileal and caecal samples showed that there were significant ($P<0.05$) differences between bacterial communities of improved performing birds (fed diets F and G) versus poorer performing birds (fed diets A and B). Within the ilea OTU 180, 188, 454, 492, 506, 566 and 938 were identified as contributing to differences in microbial composition between improved and poorer performing birds as measured by FCR. OTU 454 and 492 were more abundant in the improved performing groups, while OTU 180, 188, 506, 566 and 938 were more abundant in the lower performing groups.

Within the caeca it was found that sex of birds also significantly influenced microbial community composition, with 16 and 11 discriminating OTU identified between the improved and poorer performing birds for males and females respectively. However, for both males and females 11 common OTU (140/142, 212, 218/220 284/286, 312, 482, 488 and 536) were identified as contributing to differences in microbial composition between improved and poorer performing chickens. OTU 140/142, 218/220, 284, 312, 482, 488, and 536 were more abundant in the better performing groups, while OTU 212 was more abundant in the lower performing groups.

Chapter 5: Characterisation of performance related OTU across three broiler performance trials

Introduction

Microbial profiling was done to investigate changes in broiler gut microbiota and identify potential bacteria linked with performance (see chapters 2, 3 and 4 for more detail). In this chapter, information on potential performance related OTU is collated from all three collaborative performance trials and the common OTU characterised by nucleotide sequence determination.

Materials and methods

Cloning and sequencing operational taxonomic units

Targeted cloning and sequencing of potential performance related OTU was done as described in chapter 1.

16S rRNA sequence analysis

Determination of bacterial classification was based on generated 16S rRNA sequence information as described in chapter 1. Bacterial classification and predicted *in-silco* TR-Fs for sequences obtained in this study are shown in Appendix C.

Results

Summary of results from performance trials 1, 2 and 3

Results from all three performance trials are summarised in Table 5.1. OTU are listed where changes in either ileal and/or caecal microbial communities were detected in response to dietary treatment, and which could also be linked to broiler performance as determined by feed efficiency. Within the ilea four common OTU were identified across trials (180, 492, 564-566 and 936-938). Of these OTU 492 was consistently associated with improved performance and OTU 564-566 was associated with poorer performance. Within the caeca five common OTU were identified (140-142, 218-220, 284-286, 312 and 482) with OTU 140-142 and 482 consistently associated with improved performance.

Characterisation of potential performance related OTU

Targeted cloning and sequencing of the 16S rRNA from eight of these common OTU (180, 492, 564-566, 140-142, 218-220, 284-286, 312 and 482) revealed that they represented 22 different bacterial species. Identity of the ninth common OTU (936-938) could not be determined using our methodology despite repeated attempts. Many of the T-RFLP identified OTU contained several bacterial species which may contribute to the observed changes in performance (Table 5.2). OTU 312 (Figure 5.1), OTU 216-222 (Figure 5.2), OTU 284-286 (Figure 5.3) and OTU 482 (Figure 5.4) all contained several clusters of predicted OTU based on the obtained 16S rRNA genome information. It

is also noteworthy that even within a cluster some sequence variability existed between clones. This variability was not observed for clusters within OTU 180, 140-142 or 564-566 (data not shown).

Table 5.1: Summary of potential performance related OTU identified within the ilea and ceaca from each of three broiler performance trials.

Gut section	Trial	Association of potential performance related OTU	
		Improved performance	**Poorer performance**
Ilea	1	76, 180, 468, 492 and 936	564
	2	NS	NS
	3	454 and 492	180, 188, 506, 566 and 938
Caeca	1	NS	NS
	2	92-94, 142, 180, 198-200, 206-208, 216, 482, 542, 522 and 566	84, 144, 218, 222, 282, 284-286, 300 and 312
	3	140-142, 218-220, 284-286, 312, 482, 488 and 536	212

NS: no significant difference detected in microbial community composition between dietary treatments for which performance differences were detected.

Table 5.2: Potential performance related OTU and the determination of their bacterial classification based on 16S rRNA genome sequence information.

T-RFLP determined OTU	16S rRNA genome sequence determined OTU	Phylogenetic identification (level of classification*)
Ilea 180	181	*Lactobacillus*[#] (species)
Ilea 492	493	Unclassified *Gallibacterium* (genus)
	496	Unclassified *Enterobacteriaceae* (family)
Ilea 564-566	568	*Lactobacillus*[#] (species)
	569	*Lactobacillus*[#] (species)
	570	*Lactobacillus*[#] (species)
Caeca 140-142	144	Unclassified bacterium (domain)
Caeca 312	313-315	Unclassified bacterium (domain)
	314-316	Unclassified Clostridiales (order)
	317	Unclassified Clostridiales (order)
Caeca 216-222	210	Unclassified *Alistipes* (genus)
	216	Unclassified *Bacteroides* (genus)
	221	Unclassified *Lachnospiracaeae* (family)
	222-224	Unclassified *Lachnospiracaeae* (family)
Caeca 280-286	284-286	Unclassified *Faecalibacterium* (genus)
	287-288	Unclassified bacterium (domain)
	289	Unclassified bacterium (domain)
Caeca 482	476	Unclassified bacterium (domain)
	478	Unclassified Clostridiales (order)
	484-485	Unclassified *Lachnospiraceae* (family)
	486-487	Unclassified *Lachnospiraceae* (family)
	488	Unclassified *Lachnospiraceae* (family)

*Bacterial domain classification hierarchy: phylum, class, order, family, genus and species. Sequences were assigned a classification with a threshold of 80%.
[#]OTU 181, 568, 569 and 570 represent three different *Lactobacillus* species.

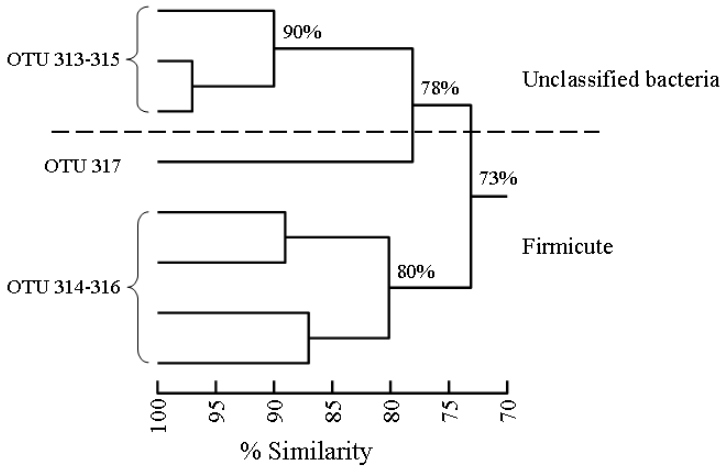

Figure 5.1: Homology tree of 16S rRNA sequences obtained from T-RFLP generated OTU 312. Separation of sequences at the phyla level is indicated by the dotted line. The dendogram is based on n=8 sequences obtained across performance trials. Separation of the T-RFLP generated OTU into predicted OTU clusters (based on genome sequence information) is also shown.

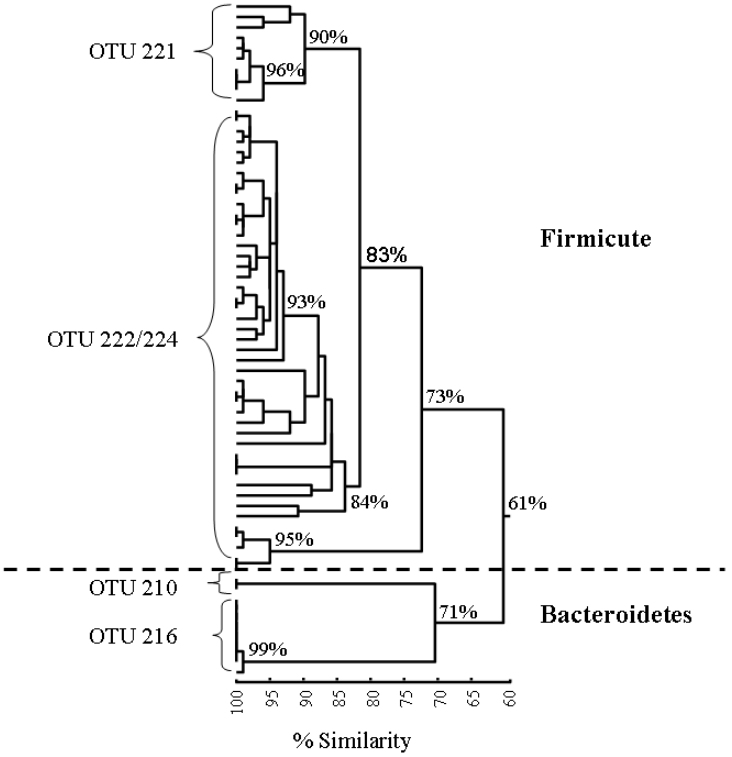

Figure 5.2: Homology tree of 16S rRNA sequences obtained from T-RFLP generated OTU 216-222. Separation of sequences at the phyla level is indicated by the dotted line. The dendogram is based on n=65 sequences obtained across performance trials. Separation of the T-RFLP generated OTU into predicted OTU clusters (based on genome sequence information) is also shown.

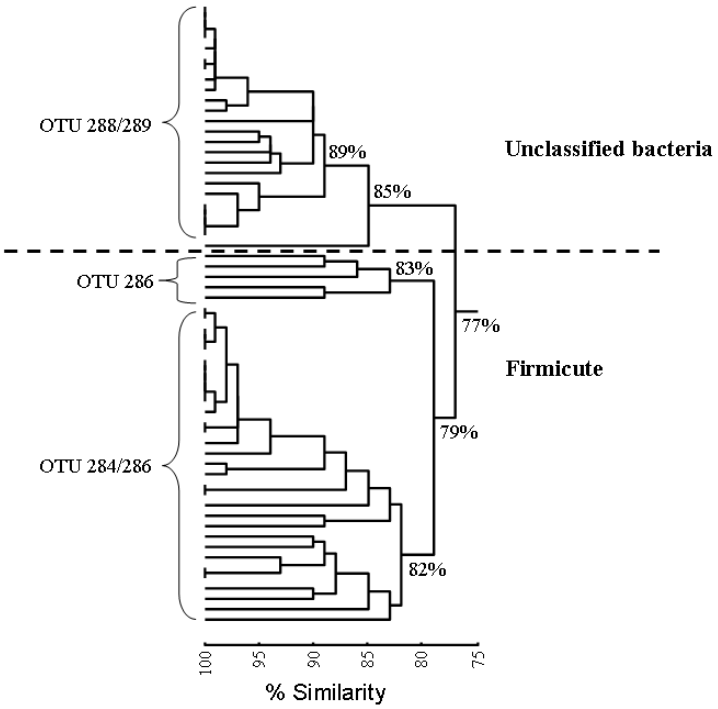

Figure 5.3: Homology tree of 16S rRNA sequences obtained from T-RFLP generated OTU 284-286. Separation of sequences at the phyla level is indicated by the dotted line. The dendogram is based on n=60 sequences obtained across performance trials. Separation of the T-RFLP generated OTU into predicted OTU clusters (based on genome sequence information) is also shown.

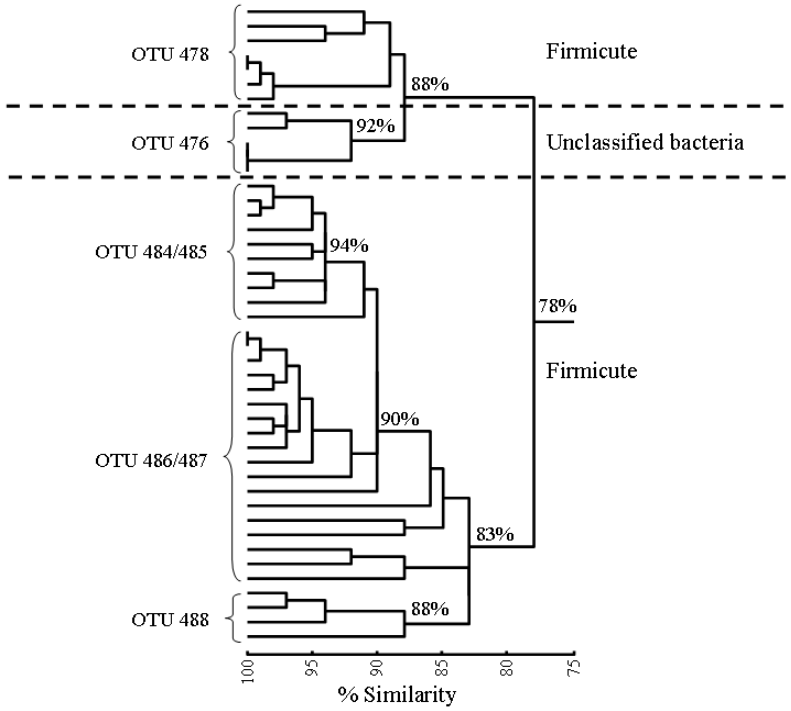

Figure 5.4: Homology tree of 16S rRNA sequences obtained from T-RFLP generated OTU 482. Separation of sequences at the phyla level is indicated by the dotted line. The dendogram is based on n=44 sequences obtained across performance trials. Separation of the T-RFLP generated OTU into predicted OTU clusters (based on genome sequence information) is also shown.

Of the 22 different phylotypes determined some were identifiable to the species level however, the majority remained unclassified bacteria. Where bacteria were identifiable to the phyla level they belonged predominantly to the Firmicutes and Bacteroidetes. Although many of these

potential performance related bacteria are unclassified, they do show high sequence similarity (90-100%) with other unclassified bacteria in public genome sequence databases (Table 5.3).

Table 5.3: Sequence identity of potential poultry performance related gut bacteria to other unclassified bacteria in GenBank. Titles of studies associated with the GenBank sequences are listed.

T-RFLP OTU	16S rRNA OTU	% Identity*	Associated study identified in GenBank
Caeca 140-142	144	95%	Symbiotic gut microbes modulate human metabolic phenotypes. (Li *et al*., 2008)
Caeca 312	313-315	97%	A core gut microbiome in obese and lean twins. (Turnbaugh *et al*., 2009)
	314-316	100%	Sequence analysis of percent G+C fraction libraries of human faecal bacterial DNA reveals a high number of Actinobacteria (Krogius-Kurikka *et al*., 2009)
	317	97%	The fecal microbiota of irritable bowel syndrome patients differs significantly from that of healthy subjects. (Kassinen *et al*., 2007)
Caeca 218-222	210	93%	Innate immunity and intestinal microbiota in the development of Type 1 diabetes. (Wen *et al.*, 2008)
	216	100%	Molecular characterization of the microbial species that colonize human ileal and colonic mucosa by using 16S rDNA sequence analysis. (Wang *et al.*, 2003)
	221	90%	Pathogen-induced host response provides competitive advantage to enteropathogens over the intestinal microbiota (Lupp *et al*., 2007; unpublished)
	222-224	99%	New profile of culturable microbiota in chicken cecum as revealed by 16S rRNA gene sequence analysis (Lan *et al*., 2006; unpublished)
Caeca 280-286	284-286	98%	Chicken intestinal microbiota modulations by various feed supplementations revealed by DGGE and CSbyDG (Massias and Urdaci, 2009; unpublished)
	287-288	94%	Potential core species and satellite species in the bacterial community within the rabbit caecum. (Monteils *et al.*, 2008)
	289	98%	A core gut microbiome in obese and lean twins. (Turnbaugh *et al*., 2009)
Caeca 482	476	96%	Evolution of mammals and their gut microbes. (Ley *et al*., 2008)
	478	99%	Succession in the intestinal microbiota of preadolescent turkeys (Scupham, 2007)
	484-485	96%	An obesity-associated gut microbiome with increased capacity for energy harvest (Turnbaugh *et al*., 2006)
	486-487	97%	New profile of culturable microbiota in chicken cecum as revealed by 16S rRNA gene sequence analysis. (Lan *et al.*, 2006; unpublished)
	488	95%	Symbiotic gut microbes modulate human metabolic phenotypes. (Li *et al*., 2008)
		95%	Characterization of mucosa adherent and invasive microbes in adenoma colorectal cancer using 16S rRNA gene profile (Shen *et al.*, 2008; unpublished)

*Sequence identity was determined in BLASTN (NCBI)

Discussion

Nine OTU were identified as being common and related to differences in broiler performance among the trials investigated. These included OTU 180, 492, 564-566, 936-938 within the ileum and OTU 140-142, 218-220, 284-286, 312 and 482 within the caeca. OTU 564-566 was predominately associated with lower performance, while OTU 492, 140-142 and 482 were predominantly associated

with improved performance. Targeted cloning and sequencing of eight of these OTU revealed that they represent 22 different bacterial species or phylotypes.

Many of the OTU identified by T-RFLP contained several bacterial species which could be contributing to the observed changes in microbial community composition. This may explain why some of the potential performance related OTU identified were not consistently associated with either improved or poorer performance across trials. For example, OTU 218-220 was more abundant in the ceaca of poorer performing birds in trial 2, whilst being more closely associated with improved performing birds in trial 3. Based on the 16S rRNA sequence information it was shown that the T-RFLP determined OTU 218-220 contained several predicted OTU (210, 216, 221 and 222-224). If one of these predicted OTU species was performance related and hypothetically decreased in abundance in improved performing birds, its response may be masked by the other OTU which could be increased in abundance due to some other factors. T-RFLP is only semi-quantitative with OTU heights indicating the amount of the organism(s) present in the bacterial community and OTU position indicating presence of a single or taxonomically related group of bacteria. Hence, if a particular OTU represents several species, the relative abundance of an individual species within the group cannot be determined. Therefore, further investigation of these predicted OTU using quantitative assays is required to determine if they are truly performance related.

From the 16S rRNA sequence information generated in this study some of the OTU were identifiable to the species level, however, the majority remained unclassified bacteria. Where bacteria were identifiable to the phyla level they belong predominantly to the Firmicutes and Bacteroidetes. Some OTU could be classified to the level of order (Clostridiales), family (*Lachnospiraceae*, *Enterobacteriaceae*), genus (*Gallibacterium*, *Alistipes*, *Bacteroides*) or even species (*Lactobacillus* sps.) The relative abundance of the Bacteroidetes and Firmicutes have been shown to differ in genetically predisposed obese versus lean mice (Turnbaugh *et al.*, 2006), indicating particular bacterial groups have increased capacity for energy harvest. Although many of our potential performance related bacteria are unclassified, they do show high sequence similarity (90-100%) with other unclassified bacteria in public genome sequence databases. Many of these similar sequences were identified from studies investigating the relationship between the gut microbiome and host metabolic phenotype, innate immunity and gut microbiota, gut microbiota in various host species including poultry and the role of gut microbiota in gut health (Kassinen *et al*., 2007; Krogius-Kurikka *et al*., 2009; Ley *at al*., 2008; Li *et al*., 2008; Monteils *et al*., 2008; Scupham, 2007; Turnbaugh *et al*., 2006; Turnbaugh *et al*., 2009; Wang *et al*., 2003; Wen *et al*., 2008). Furthermore, in this study OTU 564-566 was characterised as representing three different Lactobacillus species. This OTU was also consistently identified as being associated with decreased performance across trials. Some lactobacilli with bile deconjugating activity have previously been implicated in reduced performance in poultry (Harrow *et al*., 2007; Knarreborg *et al*., 2002a).

Overall these results are promising in our quest to identify potential performance related gut bacteria in poultry. The 16S rRNA genome sequences generated in this study also gives us the basis for developing quantitative assays to these organisms which will allow us to validate the presence of performance related gut bacteria.

Chapter 6: Development of quantitative assays for performance related bacteria

Introduction

Quantitative PCR (qPCR) assays targeting performance related OTU were designed based on 16S rRNA sequence information generated in this project. qPCR allows quantification of a target organism in an experimental sample by monitoring the fluorescence emitted during the reaction as an indicator of amplicon production at each PCR cycle. Our qPCR assays were all based on SYBR green detection, a relatively simple qPCR technology platform. SYBR green is a dye which binds double stranded DNA (dsDNA), providing a fluorescent signal that reflects the amount of dsDNA product generated during PCR. Sensitivity of our qPCR assays were tested against dilutions of known amounts of target (plasmid standards). Specificity of our qPCR assays were determined by analysing dissociation or melt curves, as well as gel electrophoresis. Specificity was further analysed for each promising qPCR assays by running it against DNA obtained from chicken gut and associated digesta (containing a mixed population of unknown bacteria) and verifying genome sequence information from the resulting amplicon.

Materials and methods

Quantitative PCR (qPCR)

Primer design and qPCR conditions

qPCR primers were designed to predicted OTU 144, 568, 569, 570, 493 and 496 based on 16S rRNA sequence information. Predicted OTU represent T-RFLP generated OTU 104-142, 564-566 and 492. Predicted OTU 568, 569 and 570 all belonged to the same genus (*Lactobacillus*) and showed a minimum of 83% sequence similarity among the three species. OTU 493 and 496 belonged to the family *Enterobacteriaceae* and showed 84% sequence similarity. Primers were designed manually to regions discriminating between related sequences from multiple sequence alignments (DNAMAN). qPCR guidelines for primer design were followed (Power SYBR Green PCR Master Mix Protocol, Applied Biosystems) and resulting primers were tested using Primer3Plus (Untergasser *et al*., 2007). All primers were designed with a Tm = 60 ± 2^{o}C so that all assays could be run under the same conditions. Designed primers were tested for specificity *in silico* against bacterial genome sequences available in public genome sequence databases (Primer-BLAST, NCBI).

qPCR was performed with 50 nM each primer and 2 x Power SYBR Green PCR Master Mix (Applied Biosystems) according to manufacturer's recommendations. Reactions were run on a 7900HT real-time PCR system (Applied Biosystems).

Creating a standard curves

Theoretically a single copy of target should create a Ct value of 40 if amplification efficiency is 100%. This is the y-intercept in a standard curve experiment. The slope of the log-linear phase is a reflection of the amplification efficiency (E (%) = ($10^{[-1/\text{slope}]}$ -1) x 100). The efficiency of the PCR should be 90-110% with an ideal slope = 3.32.

To determine the detection limit of each assay and to develop a standard curve, a known concentration of plasmid (containing cloned target sequence) was used in a series of dilutions ranging from 2 x 10^6 to 2 x 10^0 copies per µl. The reaction mixture without the plasmid DNA was used as a negative control. Plasmid standards were calculated as described in "Creating standard curves with genomic DNA or plasmid DNA templates for use in quantitative PCR" (Applied Biosystems).

Assay specificity

SYBR green is a chemical which will intercalate all double stranded DNA including non-specific amplicons and primer dimers. Therefore, for SYBR green qPCR assays a dissociation (melting) curve analysis should be performed to determine if the qPCR assay is specific to its target. The temperature-dependent dissociation between two DNA-strands can be measured and ideally should be a single peak. If multiple peaks occur then the assay is non-specific or may be forming primer dimers. If experimental samples yield a sharp peak at the desired melting temperature then the assay is a direct measure of accumulation of the product of interest.

qPCR amplicons from experimental samples were also electrophoresed on an agarose gel to visually check for a single amplicon of expected size. Amplicons of expected size were excised from the agarose gel, cloned and sequenced to confirm nucleotide sequence specificity.

Results

qPCR sensitivity

Five qPCR assays were developed to the predicted OTU (568, 569, 570, 493 and 496). A qPCR assay was not developed to OTU 144 due to limited sequence information (144 bp) for alternative primer design options. Potential primers were developed for OTU 144, however when tested *in silico* (Pimer-BLAST) they were non-specific and potentially able to detect other related gut inhabiting bacteria.

qPCR efficiency for each assay was determined (Table 6.1) and ranged from 63-97%. OTU 569 had the lowest efficiency but was the most promising assay developed for this organism when other factors, such as the dissociation curve and nucleotide sequence specificity, were taken into account.

qPCR specificity

Dissociation curve analysis of qPCR amplicons from experimental samples generated a single peak at the desired melting temperature. qPCR amplicons generated from poultry gut samples were electrophoresed on an agarose gel to confirm presence of a single amplicon of expected size. Single amplicons of expected size were confirmed for all assays (Table 6.1). qPCR amplicons produced from poultry gut samples were cloned and eight clones each were sequenced to confirm genome identity. Nucleotide sequence analysis confirmed specificity of all qPCR assays.

Table 6.1: Determination of real-time PCR efficiencies from OTU plasmid standards. Ct cycles versus plasmid copy number (log10) were plotted to calculate the slope (n = 2/assay). The corresponding real-time PCR efficiencies were calculated according to the equation: E (%) = $(10^{[-1/\text{slope}]} -1) \times 100$.

OTU	Slope	y-intercept	Efficiency (%)	Amplicon length (bp)
493	-3.502	31.34	93%	137
496	-3.782	34.27	84%	312
568	-3.506	33.12	93%	130
569	-4.744	43.23	63%	117
570	-3.390	30.82	97%	150

Discussion

Quantitative assays have been developed for potential performance related OTU 493, 496, 568, 569 and 570. All developed assays were specific to the target organism. Assay efficiency ranged from 63-97%, with OTU 569 (63%) and OTU 496 (84%) qPCR assays having the lowest amplification efficiencies. All other assays had ideal amplification efficiency and were greater than or equal to 93%. A number of variables can affect the efficiency of the qPCR and can include length of the amplicon, secondary structure and primer design.

qPCR primers were also designed to the unclassified bacterium OTU 144. But due to the limited sequence information available for primer design (144 bp) and the non-specific *in-silico* nature (primer-BLAST) of designed primers, this assay was not developed further. However, an assay is able to be developed for this organism in the future by extending the amount of genome sequence information. This can be done using PCR amplification with a specific primer designed to the currently available sequence and the T-RFLP reverse primer. This would extend sequence information toward the unknown 3' end of the genome.

Development of qPCR assays for many of the other OTU identified within the caeca was beyond the scope of this current project due to the variability in sequences obtained from T-RFLP generated OTU, high sequence similarity among predicted OTU (limiting unique primer design sites) and limited length of sequence information (200-500 bp depending on OTU size range). Ultimately, qPCR assays can be developed to these OTU, however, it will require resources outside of this project. Longer genome sequence information will be required, and qPCR assays may need to be developed based on probe based technology instead of SYBR green.

Validation of the five qPCR assays developed is still required to prove whether or not they target performance related organisms in poultry. This project has resources in the form of extensive DNA collection from a number of performance trials which can be investigated using these quantitative assays. The qPCR assays were all developed to be run under the same conditions allowing ease of use and multiple assay runs.

General discussion

Gut microbiota positively influence the host's gastrointestinal development, biochemistry, immunology, physiology, and non-specific resistance to infection (Gordon and Pesti, 1971). The initial microbiota to which chicks are exposed, as well as the nutrient composition of their diet, affect their commensal microbiota and the development of the immune system (Shira *et al.*, 2005). Colonisation of the gastrointestinal tract by bacteria can be beneficial (symbiotic), benign or detrimental (pathogenic) to the host. The use of in-feed antimicrobials in the poultry industry has played a major role in the control of pathogenic bacteria. This has had positive effects on animal welfare, animal production and economic return yet their exact mode of action has not been determined, although it is generally believed that antibiotics modulate gut microbiota and dampen immune response (Niewold, 2007). The role of commensal gut microbiota in animal production is currently receiving much interest, particularly since the withdrawal of in-feed antimicrobials in the European Union in 2006.

Many studies have investigated potential alternatives to in-feed antimicrobials. These include prebiotics, probiotics, essential oils and dietary acidifiers, yet a deeper fundamental understanding of how these compounds influence the gut microbiota, immunity, health, physiology and ultimately production traits of the bird is lacking. We have previously shown that in-feed antimicrobials alter the gut microbiota and that alternatives do not necessarily affect the gut microbial communities in the same way (Geier *et al.*, 2009; Torok, 2008). Therefore, we need to understand how antimicrobials influence gut microbiota before we can successfully find alternatives. Furthermore, we need a greater understanding of the commensal post-hatch gut microbiota development and how this affects life long health and production. Many studies investigating poultry gut microbiota are undertaken well after hatch. We have previously demonstrated that gut microbiota changes during the six week production period for broilers, with most dramatic changes observed in the first two weeks post-hatch (Torok *et al*., 2007)

In this study we characterised the normal post-hatch gut microbiota development in broiler chicks and investigated the impact of three in-feed antimicrobials on commensal gut microbiota colonisation and broiler performance. The three in-feed antimicrobials (avilamycin, flavophospholipol and zinc bacitracin) were chosen as they are currently in use within the Australian poultry industry. Each antimicrobial is reported to have a different mode of action *in vitro* and is primarily active against gram positive bacteria (Anonymous, 1997). However, their effects on overall gut microbiota *in vivo* have not been investigated in great detail.

None of the three in-feed antimicrobials investigated influenced broiler performance as measured by FCR, body weight or feed intake in the first 17 days post-hatch. Overall chick mortality ranged from 1.9 to 5.6% in the trial with the lowest observed for zinc bacitracin and highest observed for flavophospholipol. The lack of significant performance responses in this study was not entirely surprising given the small number of experimental units (n=4 pens/treatment), and the fact that a growth promotion response to in-feed antimicrobials is not always evident in highly sanitized research facility environments (Dumonceaux *et al.*, 2006; Pedroso *et al.*, 2006).

Addition of in-feed antimicrobials to the diet significantly affected gut microbiota development as compared with the control group, as well as between in-feed antimicrobial groups. The latter indicates that the three antimicrobials investigated have different modes of action *in vivo*. Flavophospholipol had the most consistent effect on gut microbial communities and was most predicable within the ilea. Avilamycin also had a more predicable impact on the ileal microbial communities. The proximal gut microbiota has been reported to be more susceptible than the distal gut to antibiotics (Dumonceaux *et al.*, 2006; Wise and Siragusa, 2007). Interestingly, in this study zinc bacitracin had least effect on the gut microbiota. Antibiotic resistance to this compound may have been a possible explanation. Indeed bacterial resistance to all three antimicrobial investigated in this study has been previously reported (Anonymous, 1997). However, this can not be confirmed as antibiotic resistance in bacteria was not investigated in our study.

We have shown that the poultry gut is rapidly colonised with a complex bacterial population. Differences in gut microbial population associated with treatment were already detectable at 3 days post-hatch. Although in-feed antimicrobials did influence the commensal gut microbiota it was interesting to note that similar temporal shifts were observed regardless of dietary composition. The ileal microbial communities showed three waves of bacterial succession (3-5 days, 5-12 days and 12-17 days post-hatch), while the caecal communities showed two waves of succession (3 days and 5-17 day post-hatch). Some common operational taxonomic units (OTU; which represent specific bacterial groups or taxonomically related groups of bacteria) were detected in both the caeca and ilea, however, the majority of OTU detected were unique to their environmental niche. The common ileal and caecal OTU suggests that the ileal communities may seed the caeca.

Most of the OTU characterised at the genome level in the first 17 days post-hatch belonged to unidentified bacteria; where phyla could be identified they belonged to Firmicutes, Bacteroidetes and Proteobacteria. Some bacteria could be further classified to the family level (*Lachnospiraceae, Lactobacillaceae*, *Enterobaceriaceae*, *Ruminococcaeceae, Bacteroidaceae* and *Oxalobacteraceae)*. In a few cases sequences could be classified to the genus level and included *Shigella, Lactobacillus* and *Lachnospiraceae Incertae Sedis*. Although most of the bacteria were unidentifiable they did show genome sequence similarity to other gastrointestinal inhabiting bacteria available in public genome sequence databases.

Members of the *Lactobacillaceae* family are prominent in the gastrointestinal following hatch (Guan *et al*., 2003). Of the seven *Lactobacillus* species (*L. avarius*, *L. acidophilus*, LCGA, *L. gasseri*, *L. johnsonii*, *L. reuteri* and *L. salivarius* subsp. *salivarius*) and two *Pediococcus* species (*P. acidilactici* and *P. pentosaceus*) investigated by Lac-PCR DGGE all were detectable to some degree. However, *P. acidilactici* was only detectable in birds aged 3 days and *L. johnsonii* and *L. reuteri* were more prevalent in the older chicks. The LCGA group was dominant in all age groups and the presence of *L. crispatus* was confirmed by 16S rRNA genome sequence information. Genome sequence information obtained from T-RFLP derived OTU data supported the presence of *L. johnsonii*, *L. crispatus* and *L. reuteri*. These findings support the autochthonous nature of these species in the chicken gastrointestinal tract, which have been reported to be present in birds of various ages (Knarreborg *et al*. 2002b; Lu *et al*., 2003; Gong *et al.*, 2008; Guan *et al.* 2003). In-feed antimicrobial treatment was shown to influence abundance of three *Lactobacillus* species.

Three broiler performance trials were investigated in an attempt to identify gut bacteria which could be consistently linked with changes in performance. Trials were done in various Australian states and varied in composition of raw dietary ingredient, dietary supplementation with various antimicrobials (antibiotics and coccidiostats) and NSP degrading enzymes, broiler breed and bird age. Trials 1 and 3 investigated birds at 42 days of age whereas trial 2 investigated birds at 35 days of age. Trials 2 and 3 were done on Cobb 500 broilers, whereas trial 1 was done on Arbor Acres broilers. Diets across trials also varied greatly. However, what was evident was that improved bird performance could be associated with a variety of dietary formulations. This was not always predicatable, for example birds on the sorghum based diets performed best in trial 1, while birds on a higher percentage sorghum diet performed worst in trial 3. NSP degrading enzymes, antibiotic and coccidiostats are all known to alter the gut bacterial populations and are often used to counteract the undesirable effects of certain raw ingredients in the broiler diet, subsequently improving performance. A variety of gut microbiota were identified as being able to able to equally maintain and promote optimal broiler performance. Gut microbiota has previously been reported to be influenced by environmental factors (litter), diet and age (Torok *et al.*, 2008; Torok *et al*., 2009). The aim of our work was not to identify changes in gut microbiota associated with diet but identify consistent changes in gut microbiota associated with performance.

Nine OTU were identified as being common and related to differences in broiler performance among the three Australian trials investigated. These included OTU 180, 492, 564-566, 936-938 in the ileum and OTU 140-142, 218-220, 284-286, 312 and 482 in the caeca. OTU 564-566 was

predominately associated with lower performance, while OTU 492, 140-142 and 482 were predominantly associated with improved performance. Targeted cloning and sequencing of eight of these OTU revealed that they represent 22 different bacterial species or phylotypes. Many of the OTU identified by T-RFLP contained several bacterial species which could be contributing to the observed changes in microbial community composition. This may explain why some of the potential performance related OTU identified were not consistently associated with either improved or poorer performance across trials. More detailed investigation is required to determine which bacterial species within an OTU are performance related.

From the 16S rRNA sequence information generated in this study some of the OTU were identifiable to the species level, however, the majority remained unclassified bacteria. Where bacteria were identifiable to the phyla level they belong predominantly to the Firmicutes and Bacteroidetes. Some OTU could be classified to the level of order (Clostridiales), family (*Lachnospiraceae, Enterobacteriaceae*), genus (*Gallibacterium, Alistipes, Bacteroides*) or even species (*Lactobacillus* sps.) The relative abundance of the Bacteroidetes and Firmicutes have been shown to differ in genetically predisposed obese versus lean mice (Turnbaugh *et al.*, 2006), indicating particular bacterial groups have increased capacity for energy harvest. Although many of our potential performance related bacteria are unclassified, they do show high sequence similarity (90-100%) with other unclassified bacteria in public genome sequence databases. Many of these similar sequences were identified from studies investigating the relationship between the gut microbiome and host metabolic phenotype, innate immunity and gut microbiota, gut microbiota in various host species including poultry and the role of gut microbiota in gut health (Kassinen *et al*., 2007; Krogius-Kurikka *et al*., 2009; Ley *at al*., 2009; Li *et al*., 2008; Monteils *et al*., 2008; Scupham, 2007; Turnbaugh *et al*., 2006; Turnbaugh *et al*., 2009; Wang *et al*., 2003; Wen *et al*., 2008). In this study OTU 564-566 was identified as being associated with decreased performance across trials and characterised as potentially representing three different *Lactobacillus* species. Lactobacilli have previously been implicated in reduced performance in poultry (Harrow *et al*., 2007; Knarreboug *et al*., 2002a).

Studies of unrelated human individuals have revealed substantial diversity in gut microbial communities (Turnbaugh *et al*., 2009). This is parallel to the high inter-bird variation (52 - 76%) observed for birds raised on the same diet and under identical environmental conditions. Yet, despite the diversity of gut bacterial assemblages it has been shown that they can yield a core microbiome at a functional level, and that deviation from this core are associated with differences in the host physiological state (Turnbaugh *et al*., 2009). Gut bacterial function was not investigated in our study but may explain why a variety of gut microbiota are able to maintain optimal performance. Indeed several gut bacteria isolated from chicken have already been shown to have various important biochemical properties. Recently *C. perfringens, Enterococcous faecium, Streptococcus bovis*, and *Bacteroides* species have been shown to have polysaccharide degrading activity against NSPs found in grain (Beckmann *et al*., 2006). Lactobacilli have various biochemical properties, including production of antibacterial compounds (de Angelis *et al*., 2006, Stern *et al.*, 2006), β-glucanase (Jonsson and Hemmingsson, 1991), and bile salt hydrolase compounds (Knarreborg *et al*., 2002a).

Research into gut microbiota and its metabolic activity is making rapid progress. New technologies such as 16S rRNA screens, metagenomics and metabolomics are shedding light on the wide diversity of the gut bacteria as well as insight into their functions. Much knowledge has already been gained into the diversity of bacteria present within the gut from 16S rRNA sequence information; however, function cannot be extrapolated from this data. New tools for investigating microbial function will allow a greater understanding of: host-microbe interaction; importance of early gut microbiota colonisation; impact of gut microbiota colonisation on immune development and long term health; and development of feeding strategies for optimal gut health.

Overall our results are promising in our quest to identify potential performance related gut bacteria in poultry. The 16S rRNA genome sequences generated in this study has established the basis for developing quantitative assays to these organisms. This ultimately will allow us to validate the presence of performance related gut bacteria. Quantitative assays have been developed for potential

performance related OTU 493, 496, 568, 569 and 570. The qPCR assays were all developed to be run under the same conditions allowing ease of use and multiple assay runs. Development of qPCR assays for many of the other OTU identified within the caeca were beyond the scope of this current project due to the variability in sequences obtained from T-RFLP generated OTU, high sequence similarity among predicted OTU and limited length of obtained sequence information (200-500 bp depending on OTU size range). qPCR assays can be developed to these OTU, however longer genome sequence information will be required. Further development of qPCR assays may also need to be based on probe technology instead of our SYBR green approach, as the former has improved specificity. Furthermore, validation of the five qPCR assays developed in this study is still required if we are to prove that they target performance related organisms in poultry.

In conclusion, our results support the fact that the gut is rapidly colonised post-hatch, as bacterial communities were detectable at 3 days post-hatch. More importantly these early (3 days post-hatch) bacterial communities were shown to be influenced by in-feed antimicrobials. This narrow window of opportunity for influencing the gut colonisation could potentially be exploited by modifying mechanical process already in place in commercial hatcheries, such as use of spray inoculation to administer a probiotic to newly hatch chicks, or *in-ovo* inoculation, to administer a prebiotic. Performance related OTU were also identified across three Australian broiler performance trials. Bacterial genome sequences information obtained from these OTU have enabled quantitative assays to be developed which will allow the validation of performed related bacteria in poultry. This technology can be developed further as a poultry specific gut bacterial chip. The outcomes of this project are key to achieving the Australian Poultry CRC objectives of: gaining a thorough understanding of the key factors influencing digestive function and gut microbiota of broiler chickens and maintaining efficient production without the use of antibiotics; and controlled microbial colonisation of the gut of newly hatched chickens to maintain a healthy gut microbiota throughout the productive life of the birds.

Implications

- A complex gut bacterial community is established by 3 days post-hatch and is influenced by dietary modifications. These primary microbial communities may already have been influenced by conditions in the hatchery, transport to the broiler farm, farm hygiene, breeder flock microbial status and nutrition. Since the gut microbiota, gut development and bird immunity are all inter-linked, the immediate post-hatch and/or *in-ovo* periods may be important for the chicks longer term gut health and development.
- Age related shifts occur in gut microbiota regardless of dietary treatment. It would appear that gut development and microbiota colonisation are linked. During the first weeks of life there is enormous growth in the chicken's GI system, far exceeding that of the other organs, and is essential if the bird is to achieve its genetic potential. The gut also has an important immunological function which is enhanced by contact with the intestinal microbiota and/or with immuno-modulating compounds in the feed. Therefore, it may be possible to accelerate this natural process by early or *in-ovo* feeding and/or controlled gut microbial colonisation to achieve a more mature bird in terms of gut microbiota, gut development and immunity. This may ultimately reflect in better bird performance and long term health status.
- The caecal microbial communities immediately post-hatch may be a subset of ileal microbial community; however, each rapidly diversifies and evolves to its own environmental niche. Caecal communities may take longer to stabilise or reach a mature community structure. Potential performance related bacteria were identified in both the ilea and caeca at 35-42 days of age. Therefore, both communities should be considered important to bird health and performance.
- Lactobacilli are important bacteria species involved in both the commensal post-hatch gut colonisation and life long performance of broilers. Prevalence of *Lactobacillus* species is influences by age and in-feed antimicrobials. Lactobacilli have previously been implicated in binding mucous and colonising the gastrointestinal tract. Furthermore, bacterial colonisation has also been indirectly shown to alter host gene expression and mucin composition. However, we have also shown that certain *Lactobacillus* species are implicated in reduced performance (feed efficiency) in broilers 35-42 days of age.
- Consistent changes in gut microbiota which are linked to broiler performance have been identified across three independent Australian trials. Nine OTU and a potential 22 bacterial species, or phylotypes, have been identified. This implies that the specific organisms, or microbial functions attributed to particular organisms, are contributing to improved or decreased productivity.
- Quantitative assays for five potential performance related bacterial phylotypes have been developed. This will enable validation of specific bacteria with changes in performance. Ultimately this will lead to more directed diagnostic approaches for indicators of broiler performance.
- 16S rRNA bacterial sequences generated for both the post-hatch gut microbiota characterisation and identification of performance related OTU can be used to develop a poultry specific gut bacterial micro-array chip, micro-fluidic card or similar assay systems.

Recommendations

- Complete the development of quantitative assays for additional 16 potential performance related OTU identified in this study. For qPCR assays to be developed, genome sequence information will need to be extended to improve diagnostic priming and/or probe site development. Quantitative assays are both a cost and time effective diagnostic method for evaluating nutritional and environmental modifications on gut microbiota and poultry performance.
- Validate presence of performance related bacteria in chicken gut samples from performance trials using developed qPCR assays. SARDI has an extensive collection of chicken gut bacterial DNA from numerous poultry trials. This is an invaluable resource for confirming association of particular bacteria with performance.
- Quantitative assays should be developed to be run as multiple, concurrent qPCR assays. This will allow uptake of the diagnostic tool by other laboratories with similar infrastructure. Real-time PCR equipment is widely available in many diagnostic laboratories.
- 16S rRNA sequences generated in this study could be used, or contribute towards, the development of a poultry specific bacterial micro-array chip. Such chips (human intestinal tract; HIT and intestinal tract; IT) have been developed for the human gastrointestinal microbiota, but are not particularly specific for other animal hosts.
- Bacterial micro-array chip or similar assay systems could be combined with other industry relevant assays, such as poultry litter organisms involved in odour or green house gas emission, poultry pathogens and zoonotic agents.
- Bacterial gene function should be investigated in more detail as it is evident that several gut microbiota can support and promoted optimal broiler performance. It is possible that a range of bacterial species have similar functions which promote health rather than solely the presence of absence of specific bacterial species.
- Early intervention strategies should be investigated further in light of the fact that a complex and changeable gut microbiota is established by 3 days post-hatch. Interventions could be *in-ovo* changes, using prebiotics, or immediate post-hatch applications with probiotic or symbiotic products.
- Microbiological isolation of performance related bacteria should be investigated to determine function. This could lead to the development of poultry specific performance related probiotic products.
- Closer coordinated investigation of host response to post-hatch gut microbial colonisation would lead to a better understanding of host-microbe interaction.

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Appendixes

Appendix A: Abbreviations

AME	apparent metabolisable energy
ANOSIM	analysis of similarity
ANOVA	analysis of variance
ANU	Australian National University
bp	base pair
CLUSTER	Hierarchical cluster analysis
CRC	Cooperative Research Centre
d	day
DGGE	denaturing gradient gel electrophoresis
dNTP	deoxynucleotide triphosphates
DNA	deoxyribonucleic acid
dsDNA	double stranded DNA
EU	European Union
FAM	6-carboxyfluorescein
FCR	feed conversion ratio
GI	gastrointestinal
GLM	general linear model
Lac-PCR DGGE	Lactobacillus specific PCR denaturing gradient gel electrophoresis
LCGA	*Lactobacillus crispatus, L. gallinarum* and/or *L amylovorous*
LSD	least significant difference
NCBI	National Centre for Biotechnology Information
nMDS	non-metric multidimensional scaling
NSP	non-starch polysaccharide
OTU	operational taxonomic units
PCR	polymerase chain reaction
PPPI	Pig and Poultry Production Institute
ppm	parts per million
PIRSA	Primary Industries and Resources of South Australia
QDPI&F	Queensland Department of Primary Industries and Fisheries
qPCR	quantitative PCR
RIRDC	Rural Industries Research and Development Corporation
rRNA	ribosomal deoxyribonucleic acid
T-RFLP	terminal restriction fragment length polymorphism

T-RF	terminal restriction fragment
SARDI	South Australian Research and Development Institute
SCFA	short chain fatty acid
SEM	standard error of the mean
SD	standard deviation
SIMPER	similarity percentages
U	units
UNE	University of New England
UV	ultra violet

Appendix B: Post-hatch bacterial classification

SARDI-ID	Taxonomical Hierarchy* (Domain, phylum, class, order, family and genus)	*Msp*I in-silico restriction fragments#
VT001-A8	Bacteria; 100%; Firmicutes; 84%; "Bacilli"; 79%; "Lactobacillales"; 78%; Lactobacillaceae; 77%; Lactobacillus; 77%	181
VT001-B11	Bacteria; 100%; Firmicutes; 82%; "Bacilli"; 81%; "Lactobacillales"; 77%; Lactobacillaceae; 77%; Lactobacillus; 77%	181
VT001-B9	Bacteria; 100%; Firmicutes; 93%; "Bacilli"; 89%; "Lactobacillales"; 83%; Lactobacillaceae; 83%; Lactobacillus; 83%	181
VT001-C12	Bacteria; 100%; Firmicutes; 91%; "Bacilli"; 89%; "Lactobacillales"; 83%; Lactobacillaceae; 82%; Lactobacillus; 82%	181
VT001-D10	Bacteria; 100%; Firmicutes; 92%; "Bacilli"; 88%; "Lactobacillales"; 87%; Lactobacillaceae; 87%; Lactobacillus; 86%	181
VT001-D6	Bacteria; 100%; Firmicutes; 86%; "Bacilli"; 81%; "Lactobacillales"; 75%; Lactobacillaceae; 70%; Lactobacillus; 70%	181
VT001-D8	Bacteria; 100%; Firmicutes; 94%; "Bacilli"; 90%; "Lactobacillales"; 89%; Lactobacillaceae; 88%; Lactobacillus; 88%	181
VT001-E10	Bacteria; 100%; Firmicutes; 88%; "Bacilli"; 85%; "Lactobacillales"; 84%; Lactobacillaceae; 84%; Lactobacillus; 84%	181
VT001-E7	Bacteria; 100%; Firmicutes; 88%; "Bacilli"; 87%; "Lactobacillales"; 84%; Lactobacillaceae; 83%; Lactobacillus; 83%	181
VT001-E8	Bacteria; 100%; Firmicutes; 93%; "Bacilli"; 93%; "Lactobacillales"; 87%; Lactobacillaceae; 86%; Lactobacillus; 86%	181
VT001-F12	Bacteria; 100%; Firmicutes; 88%; "Bacilli"; 87%; "Lactobacillales"; 84%; Lactobacillaceae; 83%; Lactobacillus; 83%	181
VT001-F7	Bacteria; 100%; Firmicutes; 91%; "Bacilli"; 89%; "Lactobacillales"; 83%; Lactobacillaceae; 82%; Lactobacillus; 82%	181
VT001-F9	Bacteria; 100%; Firmicutes; 85%; "Bacilli"; 80%; "Lactobacillales"; 75%; Lactobacillaceae; 72%; Lactobacillus; 72%	181
VT001-G5	Bacteria; 100%; Firmicutes; 91%; "Bacilli"; 89%; "Lactobacillales"; 83%; Lactobacillaceae; 82%; Lactobacillus; 82%	181
VT001-G7	Bacteria; 100%; Firmicutes; 92%; "Bacilli"; 88%; "Lactobacillales"; 87%; Lactobacillaceae; 87%; Lactobacillus; 86%	181
VT001-G8	Bacteria; 100%; Firmicutes; 78%; "Bacilli"; 74%; "Lactobacillales"; 72%; Lactobacillaceae; 71%; Lactobacillus; 71%	181
VT001-G9	Bacteria; 100%; Firmicutes; 89%; "Bacilli"; 87%; "Lactobacillales"; 83%; Lactobacillaceae; 83%; Lactobacillus; 83%	181
VT001-H8	Bacteria; 100%; Firmicutes; 85%; "Bacilli"; 80%; "Lactobacillales"; 75%; Lactobacillaceae; 72%; Lactobacillus; 72%	181
VT002-A1	Bacteria; 100%; Firmicutes; 94%; "Bacilli"; 94%; "Lactobacillales"; 88%; Lactobacillaceae; 87%; Lactobacillus; 83%	181
VT002-A3	Bacteria; 100%; Firmicutes; 91%; "Bacilli"; 89%; "Lactobacillales"; 83%; Lactobacillaceae; 82%; Lactobacillus; 82%	181
VT002-A4	Bacteria; 100%; Firmicutes; 85%; "Bacilli"; 80%; "Lactobacillales"; 75%; Lactobacillaceae; 72%; Lactobacillus; 72%	181
VT002-B11	Bacteria; 100%; Firmicutes; 78%; "Bacilli"; 75%; "Lactobacillales"; 73%; Lactobacillaceae; 73%; Lactobacillus; 73%	181
VT002-B2	Bacteria; 100%; Firmicutes; 85%; "Bacilli"; 80%; "Lactobacillales"; 75%; Lactobacillaceae; 72%; Lactobacillus; 72%	181
VT002-B4	Bacteria; 100%; Firmicutes; 85%; "Bacilli"; 80%; "Lactobacillales"; 75%; Lactobacillaceae; 72%; Lactobacillus; 72%	181
VT002-C2	Bacteria; 100%; Firmicutes; 54%; "Bacilli"; 51%; "Lactobacillales"; 44%; Lactobacillaceae; 41%; Lactobacillus; 40%	181
VT002-D7	Bacteria; 100%; Firmicutes; 85%; "Bacilli"; 80%; "Lactobacillales"; 75%; Lactobacillaceae; 72%; Lactobacillus; 72%	181
VT002-E2	Bacteria; 100%; Firmicutes; 86%; "Bacilli"; 83%; "Lactobacillales"; 80%; Lactobacillaceae; 77%; Lactobacillus; 77%	181
VT002-F1	Bacteria; 100%; Firmicutes; 91%; "Bacilli"; 89%; "Lactobacillales"; 83%; Lactobacillaceae; 82%; Lactobacillus; 82%	181
VT002-F3	Bacteria; 100%; Firmicutes; 85%; "Bacilli"; 80%; "Lactobacillales"; 75%; Lactobacillaceae; 72%; Lactobacillus; 72%	181
VT002-G4	Bacteria; 100%; Firmicutes; 89%; "Bacilli"; 87%; "Lactobacillales"; 83%; Lactobacillaceae; 83%; Lactobacillus; 83%	181
VT002-H2	Bacteria; 100%; Firmicutes; 74%; "Bacilli"; 68%; "Lactobacillales"; 61%; Lactobacillaceae; 56%; Lactobacillus; 56%	181
VT002-H5	Bacteria; 100%; Firmicutes; 91%; "Bacilli"; 89%; "Lactobacillales"; 83%; Lactobacillaceae; 82%; Lactobacillus; 82%	181
VT003-B1	Bacteria; 100%; Firmicutes; 91%; "Bacilli"; 89%; "Lactobacillales"; 83%; Lactobacillaceae; 82%; Lactobacillus; 82%	181
VT002-H1	Bacteria; 100%; Firmicutes; 88%; "Bacilli"; 83%; "Lactobacillales"; 67%; Lactobacillaceae; 66%; Lactobacillus; 60%	181

SARDI-ID	Taxonomical Hierarchy* (Domain, phylum, class, order, family and genus)	*Msp*I in-silico restriction fragments#
VT001-A10	Bacteria; 100%; Firmicutes; 96%; "Bacilli"; 95%; "Lactobacillales"; 89%; Lactobacillaceae; 86%; Lactobacillus; 77%	189
VT001-A9	Bacteria; 100%; Firmicutes; 96%; "Bacilli"; 95%; "Lactobacillales"; 89%; Lactobacillaceae; 86%; Lactobacillus; 77%	189
VT001-B8	Bacteria; 100%; Firmicutes; 87%; "Bacilli"; 86%; "Lactobacillales"; 77%; Lactobacillaceae; 73%; Lactobacillus; 58%	189
VT001-C11	Bacteria; 100%; Firmicutes; 91%; "Bacilli"; 91%; "Lactobacillales"; 86%; Lactobacillaceae; 82%; Lactobacillus; 66%	189
VT001-C5	Bacteria; 100%; Firmicutes; 97%; "Bacilli"; 94%; "Lactobacillales"; 88%; Lactobacillaceae; 83%; Lactobacillus; 74%	189
VT001-C7	Bacteria; 100%; Firmicutes; 96%; "Bacilli"; 95%; "Lactobacillales"; 89%; Lactobacillaceae; 86%; Lactobacillus; 77%	189
VT001-C8	Bacteria; 99%; Firmicutes; 90%; "Bacilli"; 90%; "Lactobacillales"; 81%; Lactobacillaceae; 75%; Lactobacillus; 59%	189
VT001-D7	Bacteria; 100%; Firmicutes; 91%; "Bacilli"; 89%; "Lactobacillales"; 81%; Lactobacillaceae; 78%; Lactobacillus; 61%	189
VT001-E5	Bacteria; 100%; Firmicutes; 96%; "Bacilli"; 95%; "Lactobacillales"; 89%; Lactobacillaceae; 86%; Lactobacillus; 77%	189
VT001-E9	Bacteria; 100%; Firmicutes; 93%; "Bacilli"; 92%; "Lactobacillales"; 84%; Lactobacillaceae; 82%; Lactobacillus; 65%	189
VT001-F10	Bacteria; 100%; Firmicutes; 96%; "Bacilli"; 95%; "Lactobacillales"; 89%; Lactobacillaceae; 86%; Lactobacillus; 77%	189
VT001-F5	Bacteria; 100%; Firmicutes; 96%; "Bacilli"; 95%; "Lactobacillales"; 89%; Lactobacillaceae; 86%; Lactobacillus; 77%	189
VT001-F6	Bacteria; 100%; Firmicutes; 94%; "Bacilli"; 90%; "Lactobacillales"; 74%; Lactobacillaceae; 69%; Lactobacillus; 53%	189
VT001-G10	Bacteria; 100%; Firmicutes; 96%; "Bacilli"; 95%; "Lactobacillales"; 89%; Lactobacillaceae; 86%; Lactobacillus; 77%	189
VT001-H10	Bacteria; 100%; Firmicutes; 96%; "Bacilli"; 95%; "Lactobacillales"; 89%; Lactobacillaceae; 86%; Lactobacillus; 77%	189
VT001-H9	Bacteria; 100%; Firmicutes; 96%; "Bacilli"; 95%; "Lactobacillales"; 89%; Lactobacillaceae; 86%; Lactobacillus; 77%	189
VT002-A5	Bacteria; 100%; Firmicutes; 96%; "Bacilli"; 95%; "Lactobacillales"; 89%; Lactobacillaceae; 86%; Lactobacillus; 77%	189
VT002-B3	Bacteria; 100%; Firmicutes; 96%; "Bacilli"; 95%; "Lactobacillales"; 89%; Lactobacillaceae; 86%; Lactobacillus; 77%	189
VT002-B5	Bacteria; 100%; Firmicutes; 88%; "Bacilli"; 87%; "Lactobacillales"; 79%; Lactobacillaceae; 77%; Lactobacillus; 60%	189
VT002-C3	Bacteria; 100%; Firmicutes; 97%; "Bacilli"; 94%; "Lactobacillales"; 88%; Lactobacillaceae; 83%; Lactobacillus; 74%	189
VT002-C6	Bacteria; 100%; Firmicutes; 97%; "Bacilli"; 94%; "Lactobacillales"; 88%; Lactobacillaceae; 83%; Lactobacillus; 74%	189
VT002-D5	Bacteria; 100%; Firmicutes; 96%; "Bacilli"; 95%; "Lactobacillales"; 89%; Lactobacillaceae; 86%; Lactobacillus; 77%	189
VT002-E3	Bacteria; 100%; Firmicutes; 89%; "Bacilli"; 86%; "Lactobacillales"; 61%; Lactobacillaceae; 60%; Lactobacillus; 49%	189
VT002-E5	Bacteria; 100%; Firmicutes; 96%; "Bacilli"; 95%; "Lactobacillales"; 89%; Lactobacillaceae; 86%; Lactobacillus; 77%	189
VT002-H3	Bacteria; 100%; Firmicutes; 96%; "Bacilli"; 95%; "Lactobacillales"; 89%; Lactobacillaceae; 86%; Lactobacillus; 77%	189
VT002-G1	Bacteria; 100%; Firmicutes; 87%; "Bacilli"; 79%; "Lactobacillales"; 69%; Lactobacillaceae; 58%; Lactobacillus; 44%	189
VT002-F6	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 99%; Clostridiales; 99%; "Ruminococcaceae"; 96%; Subdoligranulum; 61%	198
VT004-F3	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 99%; "Ruminococcaceae"; 99%; Subdoligranulum; 85%	198
VT001-C10	Bacteria; 100%; Firmicutes; 79%; "Bacilli"; 73%; "Lactobacillales"; 66%; Lactobacillaceae; 65%; Lactobacillus; 65%	201
VT002-B1	Bacteria; 100%; Firmicutes; 89%; "Bacilli"; 86%; "Lactobacillales"; 80%; Lactobacillaceae; 78%; Lactobacillus; 78%	201
VT001-A11	Bacteria; 100%; Firmicutes; 80%; "Bacilli"; 76%; "Lactobacillales"; 67%; Lactobacillaceae; 63%; Lactobacillus; 62%	201
VT001-B6	Bacteria; 100%; Firmicutes; 84%; "Bacilli"; 80%; "Lactobacillales"; 71%; Lactobacillaceae; 67%; Lactobacillus; 67%	201
VT002-F4	Bacteria; 100%; Firmicutes; 90%; "Bacilli"; 88%; "Lactobacillales"; 83%; Lactobacillaceae; 81%; Lactobacillus; 81%	201
VT002-F2	Bacteria; 100%; Firmicutes; 80%; "Bacilli"; 74%; "Lactobacillales"; 67%; Lactobacillaceae; 66%; Lactobacillus; 65%	202
VT002-E4	Bacteria; 99%; Firmicutes; 88%; "Bacilli"; 79%; "Lactobacillales"; 73%; Lactobacillaceae; 67%; Lactobacillus; 45%	209

SARDI-ID	Taxonomical Hierarchy* (Domain, phylum, class, order, family and genus)	*Msp*I in-silico restriction fragments#
VT002-C8	Bacteria; 100%; Firmicutes; 63%; "Bacilli"; 9%; "Lactobacillales"; 8%; "Aerococcaceae"; 5%; Abiotrophia; 5%	214
VT002-H8	Bacteria; 99%; Firmicutes; 95%; "Clostridia"; 95%; Clostridiales; 95%; "Lachnospiraceae"; 82%; "Lachnospiraceae Incertae Sedis"; 56%	222
VT002-D3	Bacteria; 100%; Firmicutes; 81%; "Bacilli"; 79%; "Lactobacillales"; 77%; Lactobacillaceae; 75%; Lactobacillus; 75%	225
VT002-H4	Bacteria; 97%; Firmicutes; 42%; "Bacilli"; 29%; "Lactobacillales"; 20%; "Aerococcaceae"; 4%; Abiotrophia; 2%	229
VT002-D4	Bacteria; 97%; Proteobacteria; 26%; Epsilonproteobacteria; 15%; Campylobacterales; 15%; Helicobacteraceae; 15%; Wolinella; 15%	239
VT002-B10	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 94%; Dorea; 65%	242
VT002-D11	Bacteria; 100%; Firmicutes; 85%; "Clostridia"; 84%; Clostridiales; 83%; "Lachnospiraceae"; 50%; Dorea; 10%	279
VT004-D6	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 96%	283
VT004-E6	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 96%	283
VT003-D2	Bacteria; 100%; Firmicutes; 85%; "Clostridia"; 85%; Clostridiales; 84%; "Ruminococcaceae"; 61%; Acetanaerobacterium; 18%	285
VT002-A12	Bacteria; 100%; Proteobacteria; 32%; Gammaproteobacteria; 27%; Enterobacteriales; 13%; Enterobacteriaceae; 13%; Hafnia; 9%	286
VT002-A7	Bacteria; 100%; Firmicutes; 56%; "Clostridia"; 42%; Clostridiales; 40%; Clostridiaceae; 6%; "Clostridiaceae 1"; 4%; Oxobacter; 4%	286
VT002-B12	Bacteria; 100%; Firmicutes; 58%; "Clostridia"; 46%; Clostridiales; 45%; Clostridiaceae; 5%; "Clostridiaceae 1"; 5%; Oxobacter; 3%	286
VT002-B8	Bacteria; 100%; Firmicutes; 58%; "Clostridia"; 46%; Clostridiales; 45%; Clostridiaceae; 5%; "Clostridiaceae 1"; 5%; Oxobacter; 3%	286
VT002-C7	Bacteria; 100%; Proteobacteria; 38%; Gammaproteobacteria; 27%; Enterobacteriales; 16%; Enterobacteriaceae; 16%; Hafnia; 14%	286
VT002-D8	Bacteria; 100%; Proteobacteria; 32%; Gammaproteobacteria; 27%; Enterobacteriales; 13%; Enterobacteriaceae; 13%; Hafnia; 9%	286
VT002-E6	Bacteria; 100%; Proteobacteria; 38%; Gammaproteobacteria; 27%; Enterobacteriales; 16%; Enterobacteriaceae; 16%; Hafnia; 14%	286
VT002-E7	Bacteria; 100%; Firmicutes; 58%; "Clostridia"; 46%; Clostridiales; 45%; Clostridiaceae; 5%; "Clostridiaceae 1"; 5%; Oxobacter; 3%	286
VT002-E8	Bacteria; 100%; Proteobacteria; 32%; Gammaproteobacteria; 27%; Enterobacteriales; 13%; Enterobacteriaceae; 13%; Hafnia; 9%	286
VT002-F11	Bacteria; 99%; Firmicutes; 65%; "Clostridia"; 65%; Clostridiales; 63%; Syntrophomonadaceae; 8%; Pelospora; 7%	286
VT002-F7	Bacteria; 100%; Proteobacteria; 38%; Gammaproteobacteria; 27%; Enterobacteriales; 16%; Enterobacteriaceae; 16%; Hafnia; 14%	286
VT002-G6	Bacteria; 97%; Firmicutes; 58%; "Clostridia"; 50%; Clostridiales; 46%; Clostridiaceae; 8%; "Clostridiaceae 1"; 8%; Oxobacter; 7%	286
VT002-G7	Bacteria; 100%; Proteobacteria; 29%; Gammaproteobacteria; 16%; Enterobacteriales; 7%; Enterobacteriaceae; 7%; Hafnia; 7%	286
VT002-H12	Bacteria; 99%; Proteobacteria; 39%; Gammaproteobacteria; 30%; Enterobacteriales; 19%; Enterobacteriaceae; 19%; Hafnia; 11%	286
VT002-H7	Bacteria; 100%; Firmicutes; 53%; "Clostridia"; 37%; Clostridiales; 34%; Clostridiaceae; 6%; "Clostridiaceae 1"; 6%; Oxobacter; 6%	286
VT003-A1	Bacteria; 100%; Firmicutes; 58%; "Clostridia"; 46%; Clostridiales; 45%; Clostridiaceae; 5%; "Clostridiaceae 1"; 5%; Oxobacter; 3%	286
VT003-D1	Bacteria; 97%; Proteobacteria; 36%; Gammaproteobacteria; 20%; Enterobacteriales; 15%; Enterobacteriaceae; 15%; Hafnia; 14%	286
VT002-E11	Bacteria; 100%; Firmicutes; 52%; "Clostridia"; 49%; Clostridiales; 48%; "Lachnospiraceae"; 14%; Parasporobacterium; 10%	287
VT002-F10	Bacteria; 100%; Firmicutes; 55%; "Clostridia"; 54%; Clostridiales; 53%; Syntrophomonadaceae; 13%; Pelospora; 13%	287
VT002-A11	Bacteria; 100%; Firmicutes; 46%; "Clostridia"; 46%; Clostridiales; 43%; "Ruminococcaceae"; 24%; Faecalibacterium; 12%	288
VT002-C10	Bacteria; 100%; Firmicutes; 53%; "Clostridia"; 49%; Clostridiales; 48%; Syntrophomonadaceae; 5%; Pelospora; 5%	288
VT002-C11	Bacteria; 100%; Firmicutes; 61%; "Clostridia"; 59%; Clostridiales; 55%; "Ruminococcaceae"; 14%; Faecalibacterium; 12%	288
VT005-D5	Bacteria; 100%; Firmicutes; 49%; "Clostridia"; 49%; Clostridiales; 45%; "Ruminococcaceae"; 14%; Faecalibacterium; 12%	288
VT002-E10	Bacteria; 99%; Firmicutes; 56%; "Clostridia"; 53%; Clostridiales; 52%; "Ruminococcaceae"; 15%; Faecalibacterium; 13%	288
VT002-G11	Bacteria; 100%; Firmicutes; 54%; "Clostridia"; 52%; Clostridiales; 52%; "Ruminococcaceae"; 14%; Faecalibacterium; 9%	288
VT002-G9	Bacteria; 100%; Firmicutes; 93%; "Clostridia"; 93%; Clostridiales; 91%; "Ruminococcaceae"; 74%; Acetanaerobacterium; 62%	289

SARDI-ID	Taxonomical Hierarchy* (Domain, phylum, class, order, family and genus)	*Msp*I in-silico restriction fragments#
VT005-C5	Bacteria; 100%; Firmicutes; 49%; "Clostridia"; 49%; Clostridiales; 45%; "Ruminococcaceae"; 14%; Faecalibacterium; 12%	290
VT002-D10	Bacteria; 100%; Firmicutes; 62%; "Clostridia"; 60%; Clostridiales; 56%; "Ruminococcaceae"; 14%; Faecalibacterium; 14%	291
VT002-H11	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 99%; "Ruminococcaceae"; 85%; Sporobacter; 76%	296
VT002-B9	Bacteria; 100%; Firmicutes; 88%; "Clostridia"; 88%; Clostridiales; 88%; "Ruminococcaceae"; 64%; "Ruminococcaceae Incertae Sedis"; 50%	298
VT002-E12	Bacteria; 100%; Firmicutes; 96%; "Clostridia"; 96%; Clostridiales; 96%; "Ruminococcaceae"; 74%; "Ruminococcaceae Incertae Sedis"; 69%	298
VT002-E9	Bacteria; 100%; Firmicutes; 93%; "Clostridia"; 93%; Clostridiales; 92%; "Ruminococcaceae"; 51%; Anaerotruncus; 19%	298
VT002-F8	Bacteria; 100%; Firmicutes; 77%; "Clostridia"; 76%; Clostridiales; 76%; "Ruminococcaceae"; 41%; Faecalibacterium; 27%	298
VT002-F9	Bacteria; 100%; Firmicutes; 83%; "Clostridia"; 83%; Clostridiales; 81%; "Ruminococcaceae"; 47%; "Ruminococcaceae Incertae Sedis"; 35%	298
VT002-G8	Bacteria; 100%; Firmicutes; 81%; "Clostridia"; 79%; Clostridiales; 78%; "Ruminococcaceae"; 47%; Sporobacter; 18%	298
VT003-A2	Bacteria; 99%; Firmicutes; 96%; "Clostridia"; 96%; Clostridiales; 96%; "Ruminococcaceae"; 78%; "Ruminococcaceae Incertae Sedis"; 74%	298
VT003-C2	Bacteria; 100%; Firmicutes; 96%; "Clostridia"; 96%; Clostridiales; 96%; "Ruminococcaceae"; 74%; "Ruminococcaceae Incertae Sedis"; 69%	298
VT003-B2	Bacteria; 100%; Firmicutes; 97%; "Clostridia"; 97%; Clostridiales; 96%; "Ruminococcaceae"; 91%; Anaerofilum; 37%	300
VT002-D12	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 99%; "Ruminococcaceae"; 99%; Anaerofilum; 49%	300
VT002-H6	Bacteria; 98%; Proteobacteria; 31%; Gammaproteobacteria; 16%; Enterobacteriales; 5%; Enterobacteriaceae; 5%; Hafnia; 4%	306
VT002-A10	Bacteria; 100%; Firmicutes; 59%; "Bacilli"; 18%; "Lactobacillales"; 11%; "Aerococcaceae"; 9%; Abiotrophia; 9%	309
VT002-A9	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 99%; "Lachnospiraceae"; 99%; "Lachnospiraceae Incertae Sedis"; 71%	314
VT004-F7	Bacteria; 99%; Bacteroidetes; 29%; Sphingobacteria; 26%; Sphingobacteriales; 26%; Flexibacteraceae; 26%; Leadbetterella; 21%	384
VT004-F8	Bacteria; 99%; Bacteroidetes; 29%; Sphingobacteria; 26%; Sphingobacteriales; 26%; Flexibacteraceae; 26%; Leadbetterella; 21%	384
VT003-A9	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 68%	471
VT003-E4	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 98%	471
VT003-F8	Bacteria; 100%; Proteobacteria; 98%; Gammaproteobacteria; 98%; Enterobacteriales; 98%; Enterobacteriaceae; 98%; Shigella; 88%	471
VT004-F5	Bacteria; 100%; Proteobacteria; 98%; Gammaproteobacteria; 98%; Enterobacteriales; 98%; Enterobacteriaceae; 98%; Shigella; 58%	471
VT004-H5	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 97%	471
VT003-C3	Bacteria; 100%; Proteobacteria; 77%; Gammaproteobacteria; 63%; Legionellales; 49%; Legionellaceae; 49%; Fluoribacter; 35%	471
VT004-B6	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 96%	472
VT003-F3	Bacteria; 100%; Proteobacteria; 100%; Betaproteobacteria; 96%; Burkholderiales; 96%; Oxalobacteraceae; 95%; Duganella; 41%	488
VT003-D4	Bacteria; 100%; Firmicutes; 77%; "Clostridia"; 77%; Clostridiales; 77%; Clostridiaceae; 77%; "Clostridiaceae 1"; 76%; Anaerobacter; 54%	493
VT003-H4	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Klebsiella; 95%	494
VT005-E6	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 76%; Moryella; 60%	496
VT003-A11	Bacteria; 100%; Bacteroidetes; 39%; Sphingobacteria; 36%; Sphingobacteriales; 36%; Flexibacteraceae; 34%; Leadbetterella; 31%	496
VT003-A3	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 71%	496
VT003-A5	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Escherichia; 65%	496
VT003-B8	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Escherichia; 38%	496
VT003-D8	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Escherichia; 47%	496
VT003-F10	Bacteria; 100%; Bacteroidetes; 24%; Flavobacteria; 16%; Flavobacteriales; 16%; Flavobacteriaceae; 11%; Kaistella; 10%	496
VT003-F12	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Escherichia; 87%	496

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VT003-F4	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 81%	496
VT003-H2	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 100%	496
VT003-H8	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Escherichia; 54%	496
VT004-B4	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 93%	496
VT004-D1	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 98%	496
VT004-D2	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Escherichia; 43%	496
VT004-D9	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Escherichia; 58%	496
VT004-E7	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Proteus; 100%	496
VT004-E8	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Proteus; 100%	496
VT004-E9	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 93%	496
VT004-F9	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 99%	496
VT005-A6	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 96%	496
VT005-E1	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Escherichia; 51%	496
VT003-C8	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 98%	496
VT004-B7	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 72%	499
VT004-B8	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 72%	499
VT003-E8	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 91%	516
VT004-B1	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 65%	516
VT004-E5	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 100%	516
VT005-B6	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 99%	516
VT004-A7	Bacteria; 100%; Bacteroidetes; 21%; Sphingobacteria; 21%; Sphingobacteriales; 21%; Flexibacteraceae; 21%; Leadbetterella; 21%	522
VT004-A8	Bacteria; 100%; Bacteroidetes; 21%; Sphingobacteria; 21%; Sphingobacteriales; 21%; Flexibacteraceae; 21%; Leadbetterella; 21%	522
VT003-B10	Bacteria; 100%; Firmicutes; 71%; "Clostridia"; 67%; Clostridiales; 67%; "Peptostreptococcaceae"; 6%; Sporacetigenium; 6%	525
VT004-A9	Bacteria; 100%; Bacteroidetes; 28%; Sphingobacteria; 28%; Sphingobacteriales; 28%; Flexibacteraceae; 28%; Leadbetterella; 27%	525
VT004-B9	Bacteria; 100%; Bacteroidetes; 23%; Sphingobacteria; 23%; Sphingobacteriales; 23%; Flexibacteraceae; 23%; Leadbetterella; 23%	525
VT004-H3	Bacteria; 100%; Firmicutes; 84%; "Clostridia"; 81%; Clostridiales; 81%; "Peptostreptococcaceae"; 13%; Sporacetigenium; 13%	525
VT004-H7	Bacteria; 100%; Bacteroidetes; 27%; Sphingobacteria; 26%; Sphingobacteriales; 26%; Flexibacteraceae; 26%; Leadbetterella; 25%	525
VT004-H8	Bacteria; 100%; Bacteroidetes; 27%; Sphingobacteria; 26%; Sphingobacteriales; 26%; Flexibacteraceae; 26%; Leadbetterella; 25%	525
VT004-C1	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; Clostridiaceae; 100%; "Clostridiaceae 1"; 100%; Clostridium; 87%	532
VT005-B5	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 98%; Clostridiales; 98%; "Ruminococcaceae"; 98%; Faecalibacterium; 74%	534
VT004-A1	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 97%; "Lactobacillales"; 97%; "Enterococcaceae"; 95%; Enterococcus; 92%	539
VT005-C9	Bacteria; 100%; Firmicutes; 95%; "Clostridia"; 93%; Clostridiales; 93%; "Ruminococcaceae"; 46%; Sporobacter; 9%	539
VT003-F2	Bacteria; 100%; Bacteroidetes; 100%; Sphingobacteria; 100%; Sphingobacteriales; 100%; Sphingobacteriaceae; 100%; Pedobacter; 100%	540
VT003-C4	Bacteria; 100%; Firmicutes; 100%; "Erysipelotrichi"; 89%; "Erysipelotrichales"; 89%; Erysipelotrichaceae; 89%; Erysipelotrichaceae Incertae Sedis; 87%	541
VT005-B9	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 98%; Clostridiales; 98%; "Ruminococcaceae"; 93%; Ethanoligenens; 40%	544
VT004-A3	Bacteria; 100%; Bacteroidetes; 25%; Sphingobacteria; 25%; Sphingobacteriales; 25%; Flexibacteraceae; 25%; Leadbetterella; 22%	545

SARDI-ID	Taxonomical Hierarchy* (Domain, phylum, class, order, family and genus)	*Msp*I in-silico restriction fragments#
VT004-G3	Bacteria; 100%; Bacteroidetes; 32%; Sphingobacteria; 31%; Sphingobacteriales; 31%; Flexibacteraceae; 31%; Leadbetterella; 27%	545
VT004-G4	Bacteria; 100%; Firmicutes; 91%; "Clostridia"; 91%; Clostridiales; 91%; "Lachnospiraceae"; 77%; Syntrophococcus; 16%	144, 75
VT002-B6	Bacteria; 100%; Firmicutes; 69%; "Bacilli"; 58%; "Lactobacillales"; 45%; Lactobacillaceae; 44%; Lactobacillus; 41%	181, 19
VT002-G2	Bacteria; 98%; Firmicutes; 52%; "Bacilli"; 41%; "Lactobacillales"; 33%; "Aerococcaceae"; 1%; Abiotrophia; 1%	181, 52
VT004-B5	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; Butyrivibrio; 30%	188, 144, 75, 75
VT003-H9	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 82%; Clostridiales; 82%; "Lachnospiraceae"; 77%; Oribacterium; 33%	189, 171, 140
VT004-G6	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 93%; Enterobacteriaceae; 93%; Shigella; 68%	196, 71
VT005-H8	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 100%; Subdoligranulum; 100%	198, 102
VT004-H1	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 100%; Subdoligranulum; 100%	198, 189, 102
VT003-A7	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 99%	205, 88, 51
VT003-A6	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 100%	205, 88, 52
VT003-H6	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 99%	205, 88, 52
VT003-A12	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; Coprococcus; 52%	207, 188, 75
VT002-H10	Bacteria; 100%; Firmicutes; 97%; "Clostridia"; 96%; Clostridiales; 96%; "Lachnospiraceae"; 93%; Lachnospira; 19%	208, 15
VT005-H4	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 60%	209, 188, 75, 15
VT004-H4	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 87%	222, 171, 92
VT005-C12	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 73%	222, 171, 92, 52, 35
VT002-H9	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 98%; "Lachnospiraceae Incertae Sedis"; 46%	222, 75
VT002-C9	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 99%; "Lachnospiraceae Incertae Sedis"; 86%	222, 92
VT002-D9	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 79%	222, 92
VT002-G10	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 99%; "Lachnospiraceae Incertae Sedis"; 48%	222, 92
VT004-A5	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 69%	223, 188, 75
VT004-D4	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 69%	223, 188, 75
VT004-E4	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 89%; Hespellia; 29%	223, 188, 75
VT005-C6	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 56%	224, 188, 75
VT004-D7	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 76%	224, 188, 75, 52
VT004-D8	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 76%	224, 188, 75, 52
VT004-C3	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 99%; "Ruminococcaceae"; 93%; "Ruminococcaceae Incertae Sedis"; 52%	226, 183, 17
VT005-H12	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 99%; Clostridiales; 98%; "Lachnospiraceae"; 66%; Hespellia; 45%	240, 223, 75
VT004-F6	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 100%; Subdoligranulum; 98%	242, 198, 102
VT005-G12	Bacteria; 100%; Firmicutes; 92%; "Clostridia"; 76%; Clostridiales; 76%; "Ruminococcaceae"; 73%; Papillibacter; 39%	243, 181, 124
VT004-C6	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 100%; Subdoligranulum; 100%	247, 222
VT003-B4	Bacteria; 100%; Firmicutes; 97%; "Bacilli"; 67%; "Lactobacillales"; 44%; Streptococcaceae; 26%; Lactovum; 26%	248, 219, 92
VT004-A2	Bacteria; 100%; Firmicutes; 54%; "Bacilli"; 32%; "Lactobacillales"; 30%; "Carnobacteriaceae"; 23%; "Carnobacteriaceae 1"; 23%; Dolosigranulum; 18%	249, 192
VT004-B2	Bacteria; 100%; Firmicutes; 54%; "Bacilli"; 32%; "Lactobacillales"; 30%; "Carnobacteriaceae"; 23%; "Carnobacteriaceae 1"; 23%; Dolosigranulum; 18%	249, 192
VT005-F4	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 38%	263, 207, 15

SARDI-ID	Taxonomical Hierarchy* (Domain, phylum, class, order, family and genus)	*Msp*I in-silico restriction fragments#
VT004-C7	Bacteria; 100%; Firmicutes; 100%; "Erysipelotrichi"; 94%; "Erysipelotrichales"; 94%; Erysipelotrichaceae; 94%; Erysipelotrichaceae Incertae Sedis; 81%	265, 171, 125
VT003-B11	Bacteria; 100%; Firmicutes; 81%; "Clostridia"; 79%; Clostridiales; 79%; "Lachnospiraceae"; 43%; Shuttleworthia; 24%	282, 189
VT004-G7	Bacteria; 100%; Firmicutes; 91%; "Clostridia"; 58%; Clostridiales; 51%; "Ruminococcaceae"; 30%; Acetanaerobacterium; 14%	287, 241
VT004-G8	Bacteria; 100%; Firmicutes; 91%; "Clostridia"; 58%; Clostridiales; 51%; "Ruminococcaceae"; 30%; Acetanaerobacterium; 14%	287, 241
VT005-D6	Bacteria; 100%; Firmicutes; 76%; "Clostridia"; 76%; Clostridiales; 75%; "Ruminococcaceae"; 59%; Papillibacter; 32%	287, 76
VT005-F6	Bacteria; 100%; Firmicutes; 76%; "Clostridia"; 76%; Clostridiales; 75%; "Ruminococcaceae"; 59%; Papillibacter; 32%	287, 76
VT005-G6	Bacteria; 100%; Firmicutes; 76%; "Clostridia"; 76%; Clostridiales; 75%; "Ruminococcaceae"; 59%; Papillibacter; 32%	287, 76
VT004-A6	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 88%; Oribacterium; 15%	288, 171, 17
VT005-F5	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 97%; Subdoligranulum; 80%	288, 189
VT004-C2	Bacteria; 100%; Firmicutes; 88%; "Clostridia"; 86%; Clostridiales; 81%; "Ruminococcaceae"; 61%; Faecalibacterium; 33%	288, 189
VT004-C5	Bacteria; 100%; Firmicutes; 92%; "Clostridia"; 90%; Clostridiales; 86%; "Ruminococcaceae"; 64%; Anaerotruncus; 24%	288, 189, 52
VT003-C10	Bacteria; 100%; Firmicutes; 82%; "Clostridia"; 82%; Clostridiales; 79%; "Ruminococcaceae"; 52%; Anaerotruncus; 18%	288, 189, 52, 20
VT006-B1	Bacteria; 100%; Firmicutes; 86%; "Clostridia"; 86%; Clostridiales; 80%; "Ruminococcaceae"; 62%; Faecalibacterium; 34%	288, 210
VT004-D5	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 99%; Subdoligranulum; 84%	288, 240
VT005-D12	Bacteria; 100%; Firmicutes; 98%; "Clostridia"; 98%; Clostridiales; 98%; "Ruminococcaceae"; 95%; Faecalibacterium; 90%	288, 241
VT005-A5	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 100%; Acetanaerobacterium; 54%	289, 188, 52
VT003-D10	Bacteria; 100%; Firmicutes; 84%; "Clostridia"; 82%; Clostridiales; 76%; "Ruminococcaceae"; 50%; Papillibacter; 36%	289, 189
VT003-E10	Bacteria; 100%; Firmicutes; 68%; "Clostridia"; 67%; Clostridiales; 66%; "Ruminococcaceae"; 26%; Papillibacter; 16%	289, 189
VT003-G10	Bacteria; 100%; Firmicutes; 86%; "Clostridia"; 86%; Clostridiales; 84%; "Ruminococcaceae"; 46%; Papillibacter; 27%	289, 189
VT005-G1	Bacteria; 100%; Firmicutes; 83%; "Clostridia"; 83%; Clostridiales; 78%; "Ruminococcaceae"; 49%; Papillibacter; 30%	289, 189
VT003-H10	Bacteria; 99%; Firmicutes; 77%; "Clostridia"; 76%; Clostridiales; 74%; "Ruminococcaceae"; 50%; Papillibacter; 31%	289, 190
VT005-G5	Bacteria; 100%; Firmicutes; 88%; "Clostridia"; 84%; Clostridiales; 84%; "Ruminococcaceae"; 78%; Acetanaerobacterium; 54%	289, 214
VT005-E12	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 98%; "Lachnospiraceae"; 74%; Acetitomaculum; 34%	290, 207, 15
VT005-B10	Bacteria; 100%; Firmicutes; 100%; "Erysipelotrichi"; 100%; "Erysipelotrichales"; 100%; Erysipelotrichaceae; 100%; Erysipelotrichaceae Incertae Sedis; 100%	292, 196, 52
VT004-F1	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; Moryella; 12%	297, 188
VT004-E3	Bacteria; 100%; Proteobacteria; 64%; Gammaproteobacteria; 64%; Enterobacteriales; 44%; Enterobacteriaceae; 44%; Leclercia; 11%	297, 196, 52, 17
VT005-F12	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 100%; Papillibacter; 32%	298, 225, 17
VT004-F2	Bacteria; 100%; Firmicutes; 98%; "Clostridia"; 98%; Clostridiales; 98%; "Ruminococcaceae"; 95%; "Ruminococcaceae Incertae Sedis"; 65%	298, 226, 17
VT004-H6	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 100%; "Ruminococcaceae Incertae Sedis"; 91%	298, 226, 17
VT004-E2	Bacteria; 100%; Firmicutes; 98%; "Clostridia"; 98%; Clostridiales; 98%; "Ruminococcaceae"; 95%; "Ruminococcaceae Incertae Sedis"; 65%	298, 266, 17
VT004-B3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 99%; Anaerofilum; 42%	300, 243
VT004-H2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 100%; Subdoligranulum; 21%	300, 260
VT005-G4	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 93%	314, 171
VT005-A9	Bacteria; 100%; Firmicutes; 56%; "Erysipelotrichi"; 30%; "Erysipelotrichales"; 30%; Erysipelotrichaceae; 30%; Turicibacter; 20%	341, 192
VT005-C10	Bacteria; 100%; Firmicutes; 98%; "Bacilli"; 78%; "Lactobacillales"; 72%; "Aerococcaceae"; 37%; Globicatella; 16%	364, 181
VT004-E1	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 100%	389, 181

SARDI-ID	Taxonomical Hierarchy* (Domain, phylum, class, order, family and genus)	*Msp*I in-silico restriction fragments#
VT003-G12	Bacteria; 100%; Proteobacteria; 97%; Gammaproteobacteria; 97%; Enterobacteriales; 97%; Enterobacteriaceae; 97%; Shigella; 88%	472, 52
VT003-F7	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 96%	496, 35
VT003-G7	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 62%	496, 35
VT004-G5	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Escherichia; 35%	496, 37
VT004-D3	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Escherichia; 55%	496, 52
VT003-H7	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 97%	496, 8

* 16S rRNA sequences were assigned to a taxonomical hierarchy using "Classifier: Naive Bayesian rRNA Classifier Version 2.0, July 2007". A confidence threshold of greater than or equal to 80% should be applied for a reliable classification assignment.

Where a single *Msp*I restriction fragments is presented it represents a T-RF. Where multiple *Msp*I restriction fragments are presented it is not indicated which fragment may represent the T-RF of interest. Furthermore, it is possible that the sum of the fragments represents a T-RF.

Appendix C: Performance related bacterial classification

SARDI ID	Trial	Taxonomical Hierarchy* (Domain, phylum, class, order, family and genus)	*Msp*I in-silico restriction fragment#
VT013-E12	1	Bacteria; 100%; Firmicutes; 91%; "Bacilli"; 89%; "Lactobacillales"; 83%; Lactobacillaceae; 82%; Lactobacillus; 82%	181
VT013-F5	1	Bacteria; 100%; Firmicutes; 91%; "Bacilli"; 89%; "Lactobacillales"; 83%; Lactobacillaceae; 82%; Lactobacillus; 82%	181
VT013-G12	1	Bacteria; 99%; Firmicutes; 68%; "Bacilli"; 63%; "Lactobacillales"; 58%; Lactobacillaceae; 53%; Lactobacillus; 53%	181
VT013-H12	1	Bacteria; 100%; Firmicutes; 85%; "Bacilli"; 80%; "Lactobacillales"; 75%; Lactobacillaceae; 72%; Lactobacillus; 72%	181
VT013-B7	1	Bacteria; 100%; Firmicutes; 83%; "Bacilli"; 79%; "Lactobacillales"; 77%; Lactobacillaceae; 76%; Lactobacillus; 76%	181
VT004-A12	2	Bacteria; 100%; Firmicutes; 85%; "Bacilli"; 80%; "Lactobacillales"; 75%; Lactobacillaceae; 72%; Lactobacillus; 72%	181
VT004-C12	2	Bacteria; 100%; Firmicutes; 91%; "Bacilli"; 89%; "Lactobacillales"; 83%; Lactobacillaceae; 82%; Lactobacillus; 82%	181
VT004-H11	2	Bacteria; 100%; Firmicutes; 85%; "Bacilli"; 80%; "Lactobacillales"; 75%; Lactobacillaceae; 72%; Lactobacillus; 72%	181
VT005-A1	2	Bacteria; 100%; Firmicutes; 74%; "Bacilli"; 66%; "Lactobacillales"; 46%; Lactobacillaceae; 46%; Lactobacillus; 37%	181
VT005-A7	2	Bacteria; 100%; Firmicutes; 83%; "Bacilli"; 79%; "Lactobacillales"; 77%; Lactobacillaceae; 76%; Lactobacillus; 76%	181
VT005-B7	2	Bacteria; 100%; Firmicutes; 71%; "Bacilli"; 61%; "Lactobacillales"; 52%; Lactobacillaceae; 52%; Lactobacillus; 52%	181
VT005-C8	2	Bacteria; 100%; Firmicutes; 85%; "Bacilli"; 80%; "Lactobacillales"; 75%; Lactobacillaceae; 72%; Lactobacillus; 72%	181
VT005-D7	2	Bacteria; 100%; Firmicutes; 83%; "Bacilli"; 79%; "Lactobacillales"; 77%; Lactobacillaceae; 76%; Lactobacillus; 76%	181
VT005-D8	2	Bacteria; 99%; Firmicutes; 71%; "Bacilli"; 58%; "Lactobacillales"; 52%; Lactobacillaceae; 51%; Lactobacillus; 51%	181
VT005-G8	2	Bacteria; 100%; Firmicutes; 83%; "Bacilli"; 79%; "Lactobacillales"; 77%; Lactobacillaceae; 76%; Lactobacillus; 76%	181
VT005-H6	2	Bacteria; 100%; Firmicutes; 92%; "Bacilli"; 88%; "Lactobacillales"; 84%; Lactobacillaceae; 83%; Lactobacillus; 83%	181
VT006-A8	2	Bacteria; 100%; Firmicutes; 83%; "Bacilli"; 79%; "Lactobacillales"; 77%; Lactobacillaceae; 76%; Lactobacillus; 76%	181
VT006-B8	2	Bacteria; 100%; Firmicutes; 92%; "Bacilli"; 88%; "Lactobacillales"; 84%; Lactobacillaceae; 83%; Lactobacillus; 83%	181
VT006-C8	2	Bacteria; 100%; Firmicutes; 75%; "Bacilli"; 68%; "Lactobacillales"; 66%; Lactobacillaceae; 65%; Lactobacillus; 65%	181
VT006-D8	2	Bacteria; 100%; Firmicutes; 83%; "Bacilli"; 79%; "Lactobacillales"; 77%; Lactobacillaceae; 76%; Lactobacillus; 76%	181
VT006-E7	2	Bacteria; 100%; Firmicutes; 89%; "Bacilli"; 83%; "Lactobacillales"; 77%; Lactobacillaceae; 76%; Lactobacillus; 76%	181
VT006-E8	2	Bacteria; 100%; Firmicutes; 83%; "Bacilli"; 79%; "Lactobacillales"; 77%; Lactobacillaceae; 76%; Lactobacillus; 76%	181
VT006-F7	2	Bacteria; 99%; Firmicutes; 97%; "Bacilli"; 88%; "Lactobacillales"; 85%; Lactobacillaceae; 85%; Lactobacillus; 85%	181
VT008-H4	2	Bacteria; 100%; Firmicutes; 83%; "Bacilli"; 79%; "Lactobacillales"; 77%; Lactobacillaceae; 76%; Lactobacillus; 76%	181
VT011-C1	3	Bacteria; 100%; Firmicutes; 77%; "Bacilli"; 68%; "Lactobacillales"; 60%; Lactobacillaceae; 58%; Lactobacillus; 58%	181
VT013-C10	3	Bacteria; 100%; Firmicutes; 91%; "Bacilli"; 89%; "Lactobacillales"; 83%; Lactobacillaceae; 82%; Lactobacillus; 82%	181
VT013-D10	3	Bacteria; 100%; Proteobacteria; 38%; Epsilonproteobacteria; 21%; Campylobacterales; 21%; Helicobacteraceae; 21%; Wolinella; 21%	181
VT013-G10	3	Bacteria; 100%; Firmicutes; 86%; "Bacilli"; 83%; "Lactobacillales"; 80%; Lactobacillaceae; 77%; Lactobacillus; 77%	181
VT013-H10	3	Bacteria; 100%; Firmicutes; 85%; "Bacilli"; 80%; "Lactobacillales"; 75%; Lactobacillaceae; 72%; Lactobacillus; 72%	181
VT004-A10	2	Bacteria; 100%; Firmicutes; 67%; "Clostridia"; 57%; Clostridiales; 44%; Syntrophomonadaceae; 14%; Syntrophospora; 10%	184
VT006-A11	2	Bacteria; 100%; Firmicutes; 78%; "Clostridia"; 75%; Clostridiales; 54%; Syntrophomonadaceae; 13%; Syntrophospora; 8%	184
VT006-B11	2	Bacteria; 100%; Firmicutes; 78%; "Clostridia"; 75%; Clostridiales; 54%; Syntrophomonadaceae; 13%; Syntrophospora; 8%	184
VT006-B4	2	Bacteria; 100%; Firmicutes; 78%; "Clostridia"; 75%; Clostridiales; 54%; Syntrophomonadaceae; 13%; Syntrophospora; 8%	184
VT006-D11	2	Bacteria; 100%; Firmicutes; 83%; "Clostridia"; 79%; Clostridiales; 57%; Syntrophomonadaceae; 23%; Syntrophospora; 17%	184

SARDI ID	Trial	Taxonomical Hierarchy* (Domain, phylum, class, order, family and genus)	*Msp*I in-silico restriction fragment#
VT006-G10	2	Bacteria; 100%; Firmicutes; 67%; "Clostridia"; 57%; Clostridiales; 44%; Syntrophomonadaceae; 14%; Syntrophospora; 10%	184
VT006-H10	2	Bacteria; 100%; Firmicutes; 73%; "Clostridia"; 68%; Clostridiales; 54%; Syntrophomonadaceae; 15%; Syntrophospora; 10%	184
VT010-C10	3	Bacteria; 100%; Firmicutes; 78%; "Clostridia"; 75%; Clostridiales; 54%; Syntrophomonadaceae; 13%; Syntrophospora; 8%	184
VT010-D10	3	Bacteria; 100%; Firmicutes; 78%; "Clostridia"; 75%; Clostridiales; 54%; Syntrophomonadaceae; 13%; Syntrophospora; 8%	184
VT012-B3	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 100%; Faecalibacterium; 92%	184
VT010-C6	3	Bacteria; 100%; Firmicutes; 80%; "Clostridia"; 79%; Clostridiales; 78%; "Ruminococcaceae"; 65%; Acetanaerobacterium; 55%	187
VT013-C5	1	Bacteria; 100%; Firmicutes; 97%; "Bacilli"; 94%; "Lactobacillales"; 88%; Lactobacillaceae; 83%; Lactobacillus; 74%	189
VT013-D5	1	Bacteria; 100%; Firmicutes; 97%; "Bacilli"; 94%; "Lactobacillales"; 88%; Lactobacillaceae; 83%; Lactobacillus; 74%	189
VT004-D10	2	Bacteria; 100%; Firmicutes; 96%; "Bacilli"; 95%; "Lactobacillales"; 89%; Lactobacillaceae; 86%; Lactobacillus; 77%	189
VT004-D11	2	Bacteria; 100%; Firmicutes; 96%; "Bacilli"; 95%; "Lactobacillales"; 89%; Lactobacillaceae; 86%; Lactobacillus; 77%	189
VT005-B1	2	Bacteria; 100%; Firmicutes; 96%; "Bacilli"; 95%; "Lactobacillales"; 89%; Lactobacillaceae; 86%; Lactobacillus; 77%	189
VT005-C1	2	Bacteria; 100%; Firmicutes; 96%; "Bacilli"; 95%; "Lactobacillales"; 89%; Lactobacillaceae; 86%; Lactobacillus; 77%	189
VT005-D1	2	Bacteria; 100%; Firmicutes; 96%; "Bacilli"; 95%; "Lactobacillales"; 89%; Lactobacillaceae; 86%; Lactobacillus; 77%	189
VT005-F2	2	Bacteria; 100%; Firmicutes; 96%; "Bacilli"; 95%; "Lactobacillales"; 89%; Lactobacillaceae; 86%; Lactobacillus; 77%	189
VT006-E10	2	Bacteria; 100%; Firmicutes; 86%; "Bacilli"; 82%; "Lactobacillales"; 73%; Lactobacillaceae; 67%; Lactobacillus; 51%	189
VT005-D3	2	Bacteria; 100%; Firmicutes; 82%; "Bacilli"; 77%; "Lactobacillales"; 68%; Lactobacillaceae; 61%; Lactobacillus; 36%	189
VT010-H5	3	Bacteria; 99%; Proteobacteria; 46%; Alphaproteobacteria; 25%; Sphingomonadales; 23%; Sphingomonadaceae; 23%; Sphingosinicella; 22%	192
VT010-B10	3	Bacteria; 100%; Firmicutes; 66%; "Clostridia"; 64%; Clostridiales; 64%; "Lachnospiraceae"; 62%; Dorea; 46%	195
VT005-C7	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 100%; Subdoligranulum; 81%	198
VT008-G4	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 100%; Subdoligranulum; 81%	198
VT005-B8	2	Bacteria; 100%; Firmicutes; 86%; "Bacilli"; 80%; "Lactobacillales"; 79%; Lactobacillaceae; 77%; Lactobacillus; 76%	201
VT005-F8	2	Bacteria; 100%; Firmicutes; 61%; "Bacilli"; 48%; "Lactobacillales"; 45%; Lactobacillaceae; 44%; Lactobacillus; 44%	201
VT004-C10	2	Bacteria; 100%; Firmicutes; 76%; "Clostridia"; 68%; Clostridiales; 56%; Syntrophomonadaceae; 12%; Syntrophospora; 4%	204
VT006-G7	2	Bacteria; 100%; Firmicutes; 59%; "Bacilli"; 50%; "Lactobacillales"; 45%; Lactobacillaceae; 45%; Lactobacillus; 45%	204
VT004-A11	2	Bacteria; 100%; Firmicutes; 89%; "Clostridia"; 88%; Clostridiales; 87%; "Lachnospiraceae"; 76%; Sporobacterium; 30%	205
VT005-C2	2	Bacteria; 100%; Firmicutes; 89%; "Clostridia"; 88%; Clostridiales; 86%; "Lachnospiraceae"; 84%; "Lachnospiraceae Incertae Sedis"; 32%	207
VT005-D2	2	Bacteria; 100%; Firmicutes; 95%; "Clostridia"; 95%; Clostridiales; 93%; "Lachnospiraceae"; 91%; "Lachnospiraceae Incertae Sedis"; 50%	207
VT006-F10	2	Bacteria; 100%; Firmicutes; 92%; "Clostridia"; 92%; Clostridiales; 92%; "Lachnospiraceae"; 92%; "Lachnospiraceae Incertae Sedis"; 30%	207
VT006-G5	2	Bacteria; 100%; Firmicutes; 94%; "Clostridia"; 94%; Clostridiales; 92%; "Lachnospiraceae"; 86%; "Lachnospiraceae Incertae Sedis"; 44%	207
VT010-B6	3	Bacteria; 100%; Firmicutes; 87%; "Clostridia"; 87%; Clostridiales; 87%; "Ruminococcaceae"; 81%; Acetanaerobacterium; 58%	207
VT010-C4	3	Bacteria; 100%; Firmicutes; 91%; "Clostridia"; 91%; Clostridiales; 88%; "Lachnospiraceae"; 80%; "Lachnospiraceae Incertae Sedis"; 36%	207
VT004-B12	2	Bacteria; 100%; Firmicutes; 92%; "Bacilli"; 91%; "Lactobacillales"; 81%; Lactobacillaceae; 80%; Lactobacillus; 71%	209
VT004-D12	2	Bacteria; 100%; Firmicutes; 94%; "Bacilli"; 92%; "Lactobacillales"; 89%; Lactobacillaceae; 86%; Lactobacillus; 78%	209
VT004-C11	2	Bacteria; 100%; Firmicutes; 90%; "Clostridia"; 89%; Clostridiales; 89%; "Lachnospiraceae"; 78%; Bryantella; 24%	209
VT005-A2	2	Bacteria; 100%; Firmicutes; 97%; "Clostridia"; 97%; Clostridiales; 97%; "Lachnospiraceae"; 96%; "Lachnospiraceae Incertae Sedis"; 44%	209
VT005-B3	2	Bacteria; 100%; Bacteroidetes; 99%; Bacteroidetes; 84%; Bacteroidales; 84%; Rikenellaceae; 80%; Alistipes; 77%	210

SARDI ID	Trial	Taxonomical Hierarchy* (Domain, phylum, class, order, family and genus)	*Msp*I in-silico restriction fragment#
VT010-A6	3	Bacteria; 100%; Firmicutes; 73%; "Clostridia"; 70%; Clostridiales; 67%; "Lachnospiraceae"; 47%; Lachnospira; 7%	210
VT009-A11	2	Bacteria; 100%; Firmicutes; 92%; "Clostridia"; 92%; Clostridiales; 92%; "Ruminococcaceae"; 88%; Anaerofilum; 40%	212
VT010-G3	3	Bacteria; 99%; Firmicutes; 32%; "Erysipelotrichi"; 16%; "Erysipelotrichales"; 16%; Erysipelotrichaceae; 16%; Turicibacter; 16%	213
VT008-F11	2	Bacteria; 100%; Firmicutes; 97%; "Clostridia"; 97%; Clostridiales; 96%; "Lachnospiraceae"; 94%; Dorea; 79%	221
VT010-E5	3	Bacteria; 100%; Firmicutes; 89%; "Clostridia"; 89%; Clostridiales; 88%; "Lachnospiraceae"; 80%; Lachnospira; 40%	221
VT010-E9	3	Bacteria; 100%; Firmicutes; 91%; "Clostridia"; 91%; Clostridiales; 90%; "Lachnospiraceae"; 82%; Lachnospira; 29%	221
VT010-F2	3	Bacteria; 100%; Firmicutes; 84%; "Clostridia"; 84%; Clostridiales; 84%; "Lachnospiraceae"; 68%; Lachnobacterium; 21%	221
VT010-F5	3	Bacteria; 100%; Firmicutes; 90%; "Clostridia"; 89%; Clostridiales; 89%; "Lachnospiraceae"; 71%; Lachnobacterium; 18%	221
VT010-F9	3	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 97%; "Lachnospiraceae"; 87%; Lachnobacterium; 20%	221
VT010-G1	3	Bacteria; 100%; Firmicutes; 91%; "Clostridia"; 91%; Clostridiales; 90%; "Lachnospiraceae"; 82%; Lachnospira; 29%	221
VT010-H1	3	Bacteria; 100%; Firmicutes; 95%; "Clostridia"; 95%; Clostridiales; 95%; "Lachnospiraceae"; 85%; Lachnobacterium; 24%	221
VT004-E11	2	Bacteria; 99%; Firmicutes; 75%; "Clostridia"; 70%; Clostridiales; 67%; "Ruminococcaceae"; 26%; Sporobacter; 20%	222
VT006-D10	2	Bacteria; 100%; Firmicutes; 65%; "Clostridia"; 46%; Clostridiales; 43%; "Ruminococcaceae"; 14%; Sporobacter; 11%	222
VT006-D5	2	Bacteria; 100%; Firmicutes; 61%; "Clostridia"; 56%; Clostridiales; 52%; "Ruminococcaceae"; 16%; Sporobacter; 15%	222
VT004-B10	2	Bacteria; 100%; Firmicutes; 98%; "Clostridia"; 98%; Clostridiales; 98%; "Lachnospiraceae"; 90%; Dorea; 28%	222
VT004-H10	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 99%; Dorea; 62%	222
VT005-B2	2	Bacteria; 100%; Firmicutes; 93%; "Clostridia"; 91%; Clostridiales; 90%; "Lachnospiraceae"; 74%; Dorea; 18%	222
VT005-B4	2	Bacteria; 100%; Firmicutes; 95%; "Clostridia"; 94%; Clostridiales; 92%; "Lachnospiraceae"; 89%; Lachnobacterium; 39%	222
VT010-B4	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 96%; Dorea; 50%	222
VT010-B5	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 99%; Clostridiales; 99%; "Lachnospiraceae"; 98%; "Lachnospiraceae Incertae Sedis"; 70%	222
VT010-C8	3	Bacteria; 100%; Firmicutes; 97%; "Clostridia"; 97%; Clostridiales; 97%; "Lachnospiraceae"; 81%; "Lachnospiraceae Incertae Sedis"; 56%	222
VT010-D4	3	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 99%; "Lachnospiraceae"; 99%; "Lachnospiraceae Incertae Sedis"; 87%	222
VT010-E2	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 86%	222
VT010-G5	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 90%	222
VT004-E10	2	Bacteria; 100%; Firmicutes; 97%; "Clostridia"; 96%; Clostridiales; 95%; "Lachnospiraceae"; 90%; "Lachnospiraceae Incertae Sedis"; 46%	223
VT004-F10	2	Bacteria; 100%; Firmicutes; 97%; "Clostridia"; 96%; Clostridiales; 95%; "Lachnospiraceae"; 90%; "Lachnospiraceae Incertae Sedis"; 46%	223
VT010-B1	3	Bacteria; 100%; Firmicutes; 97%; "Clostridia"; 97%; Clostridiales; 97%; "Lachnospiraceae"; 96%; "Lachnospiraceae Incertae Sedis"; 31%	223
VT010-B7	3	Bacteria; 99%; Firmicutes; 92%; "Clostridia"; 90%; Clostridiales; 82%; "Lachnospiraceae"; 67%; Lachnobacterium; 19%	223
VT004-G10	2	Bacteria; 100%; Firmicutes; 91%; "Clostridia"; 91%; Clostridiales; 91%; "Lachnospiraceae"; 87%; Bryantella; 29%	224
VT005-A3	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 96%; "Lachnospiraceae Incertae Sedis"; 41%	224
VT005-C3	2	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 99%; "Lachnospiraceae"; 93%; "Lachnospiraceae Incertae Sedis"; 31%	224
VT006-H4	2	Bacteria; 100%; Firmicutes; 98%; "Clostridia"; 98%; Clostridiales; 97%; "Lachnospiraceae"; 88%; Dorea; 20%	224
VT007-H12	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; Dorea; 65%	224
VT008-B8	2	Bacteria; 100%; Firmicutes; 98%; "Clostridia"; 97%; Clostridiales; 97%; "Lachnospiraceae"; 89%; "Lachnospiraceae Incertae Sedis"; 32%	224
VT008-C4	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 89%; Dorea; 28%	224
VT008-G5	2	Bacteria; 100%; Firmicutes; 97%; "Clostridia"; 97%; Clostridiales; 97%; "Lachnospiraceae"; 79%; Dorea; 34%	224

SARDI ID	Trial	Taxonomical Hierarchy* (Domain, phylum, class, order, family and genus)	*Msp*I in-silico restriction fragment#
VT009-C1	2	Bacteria; 100%; Firmicutes; 98%; "Clostridia"; 98%; Clostridiales; 97%; "Lachnospiraceae"; 89%; Dorea; 40%	224
VT010-A4	3	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 98%; Clostridiales; 97%; "Lachnospiraceae"; 79%; "Lachnospiraceae Incertae Sedis"; 38%	224
VT010-C7	3	Bacteria; 100%; Firmicutes; 95%; "Clostridia"; 95%; Clostridiales; 93%; "Lachnospiraceae"; 75%; Dorea; 30%	224
VT010-D5	3	Bacteria; 100%; Firmicutes; 98%; "Clostridia"; 98%; Clostridiales; 98%; "Lachnospiraceae"; 89%; Hespellia; 50%	224
VT010-D7	3	Bacteria; 100%; Firmicutes; 96%; "Clostridia"; 96%; Clostridiales; 96%; "Lachnospiraceae"; 83%; Dorea; 40%	224
VT010-H3	3	Bacteria; 100%; Firmicutes; 97%; "Clostridia"; 97%; Clostridiales; 97%; "Lachnospiraceae"; 83%; Dorea; 33%	224
VT004-G12	2	Bacteria; 100%; Firmicutes; 31%; "Clostridia"; 26%; Clostridiales; 26%; "Lachnospiraceae"; 9%; Sporobacterium; 2%	226
VT005-F3	2	Bacteria; 100%; Firmicutes; 96%; "Clostridia"; 94%; Clostridiales; 91%; "Lachnospiraceae"; 82%; "Lachnospiraceae Incertae Sedis"; 40%	227
VT007-D10	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 90%; "Lachnospiraceae Incertae Sedis"; 34%	229
VT008-A1	2	Bacteria; 100%; Firmicutes; 70%; "Clostridia"; 69%; Clostridiales; 64%; "Ruminococcaceae"; 39%; Acetanaerobacterium; 32%	235
VT004-F12	2	Bacteria; 100%; Firmicutes; 96%; "Clostridia"; 96%; Clostridiales; 96%; "Lachnospiraceae"; 85%; Dorea; 31%	238
VT007-B1	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 81%	242
VT008-B1	2	Bacteria; 99%; Firmicutes; 89%; "Clostridia"; 87%; Clostridiales; 85%; "Lachnospiraceae"; 66%; Dorea; 31%	242
VT008-B4	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 90%	242
VT008-D1	2	Bacteria; 100%; Firmicutes; 96%; "Clostridia"; 96%; Clostridiales; 95%; "Lachnospiraceae"; 87%; Dorea; 42%	242
VT008-H6	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 99%; Clostridiales; 99%; "Lachnospiraceae"; 97%; "Lachnospiraceae Incertae Sedis"; 63%	242
VT008-G1	2	Bacteria; 100%; Firmicutes; 92%; "Clostridia"; 92%; Clostridiales; 91%; "Lachnospiraceae"; 74%; Lachnospira; 21%	243
VT007-C6	2	Bacteria; 100%; Firmicutes; 98%; "Clostridia"; 98%; Clostridiales; 97%; "Lachnospiraceae"; 77%; Dorea; 30%	244
VT005-A4	2	Bacteria; 100%; Firmicutes; 95%; "Clostridia"; 94%; Clostridiales; 94%; "Lachnospiraceae"; 80%; Dorea; 28%	244
VT005-E2	2	Bacteria; 100%; Firmicutes; 97%; "Clostridia"; 97%; Clostridiales; 97%; "Lachnospiraceae"; 87%; Dorea; 14%	244
VT005-H3	2	Bacteria; 100%; Firmicutes; 98%; "Clostridia"; 98%; Clostridiales; 98%; "Lachnospiraceae"; 92%; "Lachnospiraceae Incertae Sedis"; 48%	244
VT006-E5	2	Bacteria; 100%; Firmicutes; 97%; "Clostridia"; 97%; Clostridiales; 97%; "Lachnospiraceae"; 87%; Dorea; 14%	244
VT007-B10	2	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 99%; "Lachnospiraceae"; 86%; Dorea; 44%	244
VT007-C5	2	Bacteria; 100%; Firmicutes; 97%; "Clostridia"; 97%; Clostridiales; 96%; "Lachnospiraceae"; 83%; Dorea; 48%	244
VT007-C8	2	Bacteria; 99%; Firmicutes; 89%; "Clostridia"; 88%; Clostridiales; 88%; "Lachnospiraceae"; 82%; Bryantella; 37%	244
VT007-E6	2	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 99%; "Lachnospiraceae"; 93%; Dorea; 59%	244
VT007-E8	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 89%; Dorea; 61%	244
VT008-A5	2	Bacteria; 100%; Firmicutes; 97%; "Clostridia"; 96%; Clostridiales; 96%; "Lachnospiraceae"; 75%; Dorea; 39%	244
VT008-G8	2	Bacteria; 100%; Firmicutes; 96%; "Clostridia"; 96%; Clostridiales; 95%; "Lachnospiraceae"; 82%; Dorea; 25%	244
VT009-C6	2	Bacteria; 99%; Firmicutes; 94%; "Clostridia"; 92%; Clostridiales; 89%; "Lachnospiraceae"; 80%; Dorea; 31%	244
VT009-C8	2	Bacteria; 100%; Firmicutes; 98%; "Clostridia"; 98%; Clostridiales; 97%; "Lachnospiraceae"; 87%; Hespellia; 36%	244
VT007-E10	2	Bacteria; 100%; Firmicutes; 95%; "Clostridia"; 95%; Clostridiales; 93%; "Ruminococcaceae"; 80%; Acetanaerobacterium; 33%	267
VT012-A5	3	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 100%	268
VT012-C4	3	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 100%	268
VT010-A2	3	Bacteria; 100%; Verrucomicrobia; 100%; Verrucomicrobiae; 100%; Verrucomicrobiales; 100%; Verrucomicrobiaceae; 100%; Akkermansia; 100%	270
VT010-B2	3	Bacteria; 100%; Verrucomicrobia; 100%; Verrucomicrobiae; 100%; Verrucomicrobiales; 100%; Verrucomicrobiaceae; 100%; Akkermansia; 100%	270

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VT007-H11	2	Bacteria; 96%; Firmicutes; 39%; "Bacilli"; 28%; "Lactobacillales"; 16%; "Aerococcaceae"; 3%; Abiotrophia; 3%	271
VT008-B5	2	Bacteria; 97%; Proteobacteria; 39%; Epsilonproteobacteria; 13%; Campylobacterales; 12%; Helicobacteraceae; 12%; Wolinella; 12%	271
VT011-E11	3	Bacteria; 100%; Firmicutes; 70%; "Clostridia"; 70%; Clostridiales; 69%; "Ruminococcaceae"; 27%; Anaerotruncus; 23%	272
VT007-E11	2	Bacteria; 100%; Firmicutes; 56%; "Clostridia"; 55%; Clostridiales; 53%; "Eubacteriaceae"; 11%; Pseudoramibacter; 11%	276
VT008-D12	2	Bacteria; 100%; Firmicutes; 91%; "Clostridia"; 87%; Clostridiales; 85%; "Ruminococcaceae"; 49%; "Ruminococcaceae Incertae Sedis"; 29%	283
VT007-A6	2	Bacteria; 100%; Firmicutes; 89%; "Clostridia"; 50%; Clostridiales; 50%; "Ruminococcaceae"; 46%; Ethanoligenens; 35%	284
VT008-D5	2	Bacteria; 100%; Firmicutes; 89%; "Clostridia"; 89%; Clostridiales; 88%; "Ruminococcaceae"; 74%; Anaerotruncus; 34%	284
VT009-A3	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 97%; Anaerotruncus; 46%	284
VT009-E7	2	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 99%; "Ruminococcaceae"; 98%; Subdoligranulum; 72%	284
VT008-A6	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 100%; Subdoligranulum; 70%	286
VT008-E5	2	Bacteria; 100%; Firmicutes; 84%; "Clostridia"; 84%; Clostridiales; 84%; "Ruminococcaceae"; 51%; Faecalibacterium; 41%	286
VT008-E8	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 100%; Faecalibacterium; 100%	286
VT008-F5	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 100%; Faecalibacterium; 100%	286
VT008-G6	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 100%; Faecalibacterium; 100%	286
VT008-G9	2	Bacteria; 100%; Firmicutes; 93%; "Clostridia"; 92%; Clostridiales; 92%; "Ruminococcaceae"; 77%; Acetanaerobacterium; 22%	286
VT009-G5	2	Bacteria; 100%; Firmicutes; 97%; "Clostridia"; 97%; Clostridiales; 97%; "Ruminococcaceae"; 93%; Anaerotruncus; 41%	286
VT010-D3	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 100%; Faecalibacterium; 100%	286
VT010-E8	3	Bacteria; 100%; Firmicutes; 88%; "Clostridia"; 88%; Clostridiales; 88%; "Ruminococcaceae"; 80%; Faecalibacterium; 64%	286
VT010-F8	3	Bacteria; 100%; Firmicutes; 84%; "Clostridia"; 82%; Clostridiales; 81%; "Ruminococcaceae"; 46%; Subdoligranulum; 14%	286
VT010-G2	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 100%; Faecalibacterium; 100%	286
VT012-C1	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 99%; Faecalibacterium; 99%	286
VT008-B6	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 100%; Faecalibacterium; 100%	287
VT010-A9	3	Bacteria; 100%; Firmicutes; 66%; "Clostridia"; 66%; Clostridiales; 66%; "Lachnospiraceae"; 15%; Parasporobacterium; 11%	287
VT010-C5	3	Bacteria; 100%; Firmicutes; 79%; "Clostridia"; 79%; Clostridiales; 75%; "Ruminococcaceae"; 40%; Faecalibacterium; 31%	287
VT010-E3	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 100%; Faecalibacterium; 100%	287
VT010-F3	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 100%; Faecalibacterium; 100%	287
VT010-C3	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 100%; Faecalibacterium; 99%	287
VT007-B7	2	Bacteria; 100%; Firmicutes; 73%; "Clostridia"; 72%; Clostridiales; 71%; "Ruminococcaceae"; 32%; Acetanaerobacterium; 9%	288
VT008-A2	2	Bacteria; 100%; Firmicutes; 54%; "Clostridia"; 52%; Clostridiales; 52%; "Eubacteriaceae"; 29%; Pseudoramibacter; 29%	288
VT008-A3	2	Bacteria; 100%; Firmicutes; 71%; "Clostridia"; 63%; Clostridiales; 60%; "Ruminococcaceae"; 27%; Acetanaerobacterium; 12%	288
VT008-B3	2	Bacteria; 100%; Firmicutes; 53%; "Clostridia"; 45%; Clostridiales; 44%; Peptococcaceae; 14%; Peptococcaceae 1; 14%; Peptococcus; 14%	288
VT008-C1	2	Bacteria; 100%; Firmicutes; 61%; "Clostridia"; 48%; Clostridiales; 48%; Peptococcaceae; 14%; Peptococcaceae 1; 14%; Peptococcus; 14%	288
VT008-C2	2	Bacteria; 100%; Bacteroidetes; 29%; Bacteroidetes; 15%; Bacteroidales; 15%; Bacteroidaceae; 14%; Megamonas; 14%	288
VT008-D2	2	Bacteria; 100%; Firmicutes; 68%; "Clostridia"; 65%; Clostridiales; 64%; "Ruminococcaceae"; 31%; Papillibacter; 10%	288
VT008-F3	2	Bacteria; 100%; Firmicutes; 65%; "Clostridia"; 57%; Clostridiales; 55%; Peptococcaceae; 10%; Peptococcaceae 1; 10%; Peptococcus; 10%	288
VT008-H1	2	Bacteria; 100%; Firmicutes; 65%; "Clostridia"; 57%; Clostridiales; 55%; Peptococcaceae; 10%; Peptococcaceae 1; 10%; Peptococcus; 10%	288

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VT008-H2	2	Bacteria; 100%; Bacteroidetes; 25%; Bacteroidetes; 8%; Bacteroidales; 8%; Bacteroidaceae; 6%; Megamonas; 6%	288
VT009-E10	2	Bacteria; 100%; Firmicutes; 74%; "Clostridia"; 73%; Clostridiales; 70%; "Ruminococcaceae"; 50%; Faecalibacterium; 24%	288
VT010-A5	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 100%; Faecalibacterium; 100%	288
VT010-D1	3	Bacteria; 100%; Firmicutes; 65%; "Clostridia"; 57%; Clostridiales; 55%; Peptococcaceae; 10%; Peptococcaceae 1; 10%; Peptococcus; 10%	288
VT010-D6	3	Bacteria; 100%; Firmicutes; 52%; "Clostridia"; 51%; Clostridiales; 51%; Peptococcaceae; 18%; Peptococcaceae 1; 17%; Peptococcus; 11%	288
VT010-G4	3	Bacteria; 100%; Firmicutes; 58%; "Clostridia"; 50%; Clostridiales; 48%; Peptococcaceae; 17%; Peptococcaceae 1; 17%; Peptococcus; 16%	288
VT007-A11	2	Bacteria; 100%; Firmicutes; 59%; "Clostridia"; 57%; Clostridiales; 55%; "Eubacteriaceae"; 24%; Pseudoramibacter; 24%	289
VT007-C10	2	Bacteria; 100%; Firmicutes; 53%; "Clostridia"; 51%; Clostridiales; 50%; "Eubacteriaceae"; 18%; Pseudoramibacter; 18%	289
VT008-E4	2	Bacteria; 99%; Bacteroidetes; 30%; Sphingobacteria; 27%; Sphingobacteriales; 27%; Flexibacteraceae; 26%; Spirosoma; 20%	289
VT008-H10	2	Bacteria; 100%; Firmicutes; 83%; "Clostridia"; 83%; Clostridiales; 83%; "Ruminococcaceae"; 74%; Acetanaerobacterium; 64%	289
VT009-D9	2	Bacteria; 100%; Firmicutes; 59%; "Clostridia"; 58%; Clostridiales; 57%; Peptococcaceae; 43%; Peptococcaceae 1; 43%; Peptococcus; 42%	289
VT010-E1	3	Bacteria; 100%; Firmicutes; 84%; "Clostridia"; 82%; Clostridiales; 82%; "Ruminococcaceae"; 69%; Papillibacter; 33%	289
VT010-F1	3	Bacteria; 100%; Firmicutes; 84%; "Clostridia"; 82%; Clostridiales; 82%; "Ruminococcaceae"; 69%; Papillibacter; 33%	289
VT007-D11	2	Bacteria; 99%; Firmicutes; 55%; "Clostridia"; 52%; Clostridiales; 49%; "Eubacteriaceae"; 12%; Pseudoramibacter; 12%	292
VT009-B10	2	Bacteria; 99%; Firmicutes; 96%; "Clostridia"; 96%; Clostridiales; 96%; "Ruminococcaceae"; 73%; Anaerotruncus; 49%	295
VT007-D2	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 98%; "Lachnospiraceae Incertae Sedis"; 42%	296
VT007-D3	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 97%; "Lachnospiraceae Incertae Sedis"; 40%	296
VT010-B9	3	Bacteria; 100%; Firmicutes; 92%; "Clostridia"; 90%; Clostridiales; 90%; "Ruminococcaceae"; 53%; Sporobacter; 23%	296
VT010-C9	3	Bacteria; 100%; Firmicutes; 92%; "Clostridia"; 92%; Clostridiales; 92%; "Ruminococcaceae"; 54%; Sporobacter; 17%	296
VT010-D9	3	Bacteria; 100%; Firmicutes; 91%; "Clostridia"; 91%; Clostridiales; 91%; "Ruminococcaceae"; 59%; Sporobacter; 32%	296
VT010-F6	3	Bacteria; 100%; Firmicutes; 83%; "Clostridia"; 83%; Clostridiales; 80%; "Ruminococcaceae"; 39%; Faecalibacterium; 23%	296
VT010-H8	3	Bacteria; 100%; Firmicutes; 91%; "Clostridia"; 90%; Clostridiales; 90%; "Ruminococcaceae"; 55%; Sporobacter; 20%	296
VT008-B9	2	Bacteria; 100%; Firmicutes; 89%; "Clostridia"; 89%; Clostridiales; 89%; "Ruminococcaceae"; 41%; Faecalibacterium; 19%	297
VT009-F9	2	Bacteria; 100%; Firmicutes; 75%; "Clostridia"; 75%; Clostridiales; 75%; "Ruminococcaceae"; 42%; "Ruminococcaceae Incertae Sedis"; 30%	297
VT007-B11	2	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 99%; "Ruminococcaceae"; 99%; Anaerotruncus; 55%	298
VT007-B4	2	Bacteria; 100%; Firmicutes; 92%; "Clostridia"; 91%; Clostridiales; 91%; "Ruminococcaceae"; 74%; "Ruminococcaceae Incertae Sedis"; 64%	298
VT007-F2	2	Bacteria; 99%; Firmicutes; 69%; "Clostridia"; 69%; Clostridiales; 69%; "Lachnospiraceae"; 17%; Parasporobacterium; 11%	298
VT007-G11	2	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 98%; "Ruminococcaceae"; 97%; Acetanaerobacterium; 39%	298
VT007-G2	2	Bacteria; 99%; Firmicutes; 77%; "Clostridia"; 75%; Clostridiales; 75%; "Ruminococcaceae"; 35%; Sporobacter; 7%	298
VT007-G3	2	Bacteria; 99%; Firmicutes; 79%; "Clostridia"; 79%; Clostridiales; 78%; "Ruminococcaceae"; 29%; Sporobacter; 13%	298
VT008-A8	2	Bacteria; 100%; Firmicutes; 91%; "Clostridia"; 91%; Clostridiales; 89%; "Ruminococcaceae"; 46%; "Ruminococcaceae Incertae Sedis"; 26%	298
VT008-B7	2	Bacteria; 100%; Firmicutes; 86%; "Clostridia"; 86%; Clostridiales; 81%; "Ruminococcaceae"; 47%; "Ruminococcaceae Incertae Sedis"; 27%	298
VT008-C12	2	Bacteria; 100%; Firmicutes; 85%; "Clostridia"; 84%; Clostridiales; 83%; Incertae Sedis XI; 17%; Finegoldia; 17%	298
VT008-D7	2	Bacteria; 99%; Firmicutes; 75%; "Clostridia"; 75%; Clostridiales; 75%; "Ruminococcaceae"; 54%; Papillibacter; 31%	298
VT008-E9	2	Bacteria; 100%; Firmicutes; 91%; "Clostridia"; 91%; Clostridiales; 89%; "Ruminococcaceae"; 46%; "Ruminococcaceae Incertae Sedis"; 26%	298
VT009-D7	2	Bacteria; 100%; Firmicutes; 81%; "Clostridia"; 81%; Clostridiales; 81%; "Ruminococcaceae"; 45%; "Ruminococcaceae Incertae Sedis"; 28%	298

SARDI ID	Trial	Taxonomical Hierarchy* (Domain, phylum, class, order, family and genus)	*Msp*I in-silico restriction fragment#
VT009-G1	2	Bacteria; 100%; Firmicutes; 92%; "Clostridia"; 92%; Clostridiales; 92%; "Ruminococcaceae"; 57%; "Ruminococcaceae Incertae Sedis"; 47%	298
VT010-G12	2	Bacteria; 100%; Firmicutes; 95%; "Clostridia"; 95%; Clostridiales; 95%; "Ruminococcaceae"; 65%; "Ruminococcaceae Incertae Sedis"; 58%	298
VT010-E6	3	Bacteria; 100%; Firmicutes; 89%; "Clostridia"; 82%; Clostridiales; 80%; "Lachnospiraceae"; 78%; "Lachnospiraceae Incertae Sedis"; 41%	298
VT010-F4	3	Bacteria; 100%; Firmicutes; 93%; "Clostridia"; 91%; Clostridiales; 89%; "Ruminococcaceae"; 61%; "Ruminococcaceae Incertae Sedis"; 47%	298
VT011-C12	3	Bacteria; 100%; Firmicutes; 81%; "Clostridia"; 80%; Clostridiales; 80%; Incertae Sedis XI; 15%; Finegoldia; 15%	298
VT011-F9	3	Bacteria; 100%; Firmicutes; 96%; "Clostridia"; 94%; Clostridiales; 94%; "Ruminococcaceae"; 62%; "Ruminococcaceae Incertae Sedis"; 58%	298
VT012-F3	3	Bacteria; 100%; Firmicutes; 76%; "Clostridia"; 71%; Clostridiales; 67%; Incertae Sedis XI; 11%; Finegoldia; 11%	298
VT012-G3	3	Bacteria; 100%; Firmicutes; 84%; "Clostridia"; 83%; Clostridiales; 83%; "Lachnospiraceae"; 21%; Lachnospira; 9%	298
VT007-F3	2	Bacteria; 99%; Firmicutes; 71%; "Clostridia"; 70%; Clostridiales; 66%; "Lachnospiraceae"; 28%; Parasporobacterium; 18%	299
VT011-D10	3	Bacteria; 100%; Firmicutes; 94%; "Clostridia"; 93%; Clostridiales; 93%; "Ruminococcaceae"; 64%; Sporobacter; 36%	299
VT012-G2	3	Bacteria; 100%; Firmicutes; 93%; "Clostridia"; 85%; Clostridiales; 85%; "Ruminococcaceae"; 69%; Sporobacter; 47%	299
VT010-B3	3	Bacteria; 100%; Firmicutes; 88%; "Clostridia"; 88%; Clostridiales; 85%; "Ruminococcaceae"; 69%; Faecalibacterium; 43%	299
VT008-B2	2	Bacteria; 100%; Firmicutes; 98%; "Clostridia"; 98%; Clostridiales; 96%; "Ruminococcaceae"; 79%; Papillibacter; 59%	300
VT010-A3	3	Bacteria; 100%; Firmicutes; 98%; "Clostridia"; 98%; Clostridiales; 97%; "Ruminococcaceae"; 96%; Anaerofilum; 35%	300
VT010-H4	3	Bacteria; 100%; Firmicutes; 96%; "Clostridia"; 95%; Clostridiales; 95%; "Ruminococcaceae"; 86%; Papillibacter; 73%	300
VT008-C11	2	Bacteria; 100%; Firmicutes; 88%; "Clostridia"; 88%; Clostridiales; 88%; "Ruminococcaceae"; 35%; Faecalibacterium; 7%	301
VT009-F12	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 100%; Faecalibacterium; 100%	301
VT008-E3	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 100%; Subdoligranulum; 87%	302
VT010-H2	3	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 99%; "Ruminococcaceae"; 99%; Subdoligranulum; 89%	302
VT009-A10	2	Bacteria; 99%; Firmicutes; 83%; "Clostridia"; 83%; Clostridiales; 83%; "Ruminococcaceae"; 47%; "Ruminococcaceae Incertae Sedis"; 21%	303
VT007-C3	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 99%; "Ruminococcaceae"; 98%; Anaerotruncus; 38%	303
VT007-C7	2	Bacteria; 100%; Firmicutes; 98%; "Clostridia"; 98%; Clostridiales; 98%; "Ruminococcaceae"; 93%; Acetanaerobacterium; 40%	303
VT007-D7	2	Bacteria; 100%; Firmicutes; 96%; "Clostridia"; 96%; Clostridiales; 95%; "Ruminococcaceae"; 93%; Anaerotruncus; 52%	303
VT007-G1	2	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 99%; "Ruminococcaceae"; 88%; Subdoligranulum; 34%	303
VT008-C7	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 99%; "Ruminococcaceae"; 98%; Anaerotruncus; 38%	303
VT008-E1	2	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 98%; Clostridiales; 97%; "Ruminococcaceae"; 89%; Anaerotruncus; 40%	303
VT008-E2	2	Bacteria; 100%; Firmicutes; 69%; "Clostridia"; 60%; Clostridiales; 60%; Peptococcaceae; 19%; Peptococcaceae 1; 19%; Peptococcus; 19%	303
VT008-E6	2	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 98%; "Ruminococcaceae"; 97%; Acetanaerobacterium; 34%	303
VT008-F6	2	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 98%; "Ruminococcaceae"; 97%; Acetanaerobacterium; 34%	303
VT008-F7	2	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 98%; "Ruminococcaceae"; 97%; Acetanaerobacterium; 34%	303
VT008-G2	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 99%; "Ruminococcaceae"; 98%; Anaerotruncus; 38%	303
VT009-B7	2	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 99%; "Ruminococcaceae"; 97%; Anaerotruncus; 61%	303
VT009-C10	2	Bacteria; 100%; Firmicutes; 98%; "Clostridia"; 98%; Clostridiales; 97%; "Ruminococcaceae"; 96%; Anaerotruncus; 51%	303
VT009-G9	2	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 97%; "Ruminococcaceae"; 89%; Acetanaerobacterium; 33%	303
VT007-F12	2	Bacteria; 100%; Firmicutes; 97%; "Clostridia"; 96%; Clostridiales; 95%; "Ruminococcaceae"; 93%; Anaerotruncus; 38%	305
VT007-A9	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 100%; Faecalibacterium; 99%	306

SARDI ID	Trial	Taxonomical Hierarchy* (Domain, phylum, class, order, family and genus)	*Msp*I in-silico restriction fragment#
VT007-B9	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 100%; Faecalibacterium; 100%	306
VT008-G7	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 100%; Faecalibacterium; 99%	306
VT008-D4	2	Bacteria; 100%; Firmicutes; 65%; "Clostridia"; 62%; "Thermoanaerobacterales"; 18%; Incertae Sedis IV; 17%; Mahella; 17%	306
VT007-D1	2	Bacteria; 100%; Firmicutes; 97%; "Clostridia"; 97%; Clostridiales; 97%; "Ruminococcaceae"; 95%; Faecalibacterium; 37%	308
VT008-D3	2	Bacteria; 100%; Firmicutes; 98%; "Clostridia"; 98%; Clostridiales; 97%; "Ruminococcaceae"; 96%; Anaerotruncus; 47%	308
VT008-H3	2	Bacteria; 100%; Firmicutes; 52%; "Clostridia"; 52%; Clostridiales; 49%; "Ruminococcaceae"; 16%; Faecalibacterium; 8%	308
VT008-F1	2	Bacteria; 99%; Firmicutes; 62%; "Clostridia"; 61%; Clostridiales; 61%; "Eubacteriaceae"; 27%; Pseudoramibacter; 27%	309
VT007-F11	2	Bacteria; 100%; Firmicutes; 42%; "Clostridia"; 40%; Clostridiales; 40%; Peptococcaceae; 20%; Peptococcaceae 1; 20%; Peptococcus; 19%	313
VT009-E9	2	Bacteria; 99%; Firmicutes; 53%; "Clostridia"; 49%; Clostridiales; 49%; Peptococcaceae; 24%; Peptococcaceae 1; 24%; Peptococcus; 24%	313
VT008-H5	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 96%; Dorea; 38%	314
VT008-H7	2	Bacteria; 100%; Firmicutes; 84%; "Clostridia"; 83%; Clostridiales; 83%; "Lachnospiraceae"; 73%; Lachnospira; 44%	314
VT010-E4	3	Bacteria; 100%; Firmicutes; 75%; "Clostridia"; 74%; Clostridiales; 74%; Peptococcaceae; 36%; Peptococcaceae 1; 36%; Peptococcus; 36%	315
VT009-E1	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 99%; Clostridiales; 99%; "Lachnospiraceae"; 99%; "Lachnospiraceae Incertae Sedis"; 63%	316
VT007-E2	2	Bacteria; 100%; Firmicutes; 90%; "Clostridia"; 90%; Clostridiales; 88%; "Ruminococcaceae"; 63%; "Ruminococcaceae Incertae Sedis"; 36%	317
VT007-E3	2	Bacteria; 100%; Firmicutes; 90%; "Clostridia"; 90%; Clostridiales; 88%; "Ruminococcaceae"; 63%; "Ruminococcaceae Incertae Sedis"; 36%	317
VT008-G12	2	Bacteria; 100%; Firmicutes; 98%; "Clostridia"; 97%; Clostridiales; 94%; "Ruminococcaceae"; 64%; Papillibacter; 36%	317
VT008-G3	2	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 99%; "Lachnospiraceae"; 98%; "Lachnospiraceae Incertae Sedis"; 71%	319
VT007-A5	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 94%; Acetanaerobacterium; 38%	323
VT008-E7	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 98%; "Ruminococcaceae"; 95%; Anaerotruncus; 36%	323
VT009-A9	2	Bacteria; 100%; Firmicutes; 96%; "Clostridia"; 96%; Clostridiales; 96%; "Lachnospiraceae"; 87%; Coprococcus; 34%	354
VT008-D11	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 61%	368
VT009-H9	2	Bacteria; 99%; Firmicutes; 63%; "Clostridia"; 63%; Clostridiales; 63%; Peptococcaceae; 34%; Peptococcaceae 1; 34%; Peptococcus; 34%	369
VT009-A6	2	Bacteria; 100%; Firmicutes; 93%; "Clostridia"; 93%; Clostridiales; 90%; "Ruminococcaceae"; 83%; Ethanoligenens; 51%	386
VT009-A7	2	Bacteria; 100%; Firmicutes; 86%; "Clostridia"; 86%; Clostridiales; 84%; "Ruminococcaceae"; 78%; Ethanoligenens; 39%	386
VT009-C3	2	Bacteria; 100%; Firmicutes; 83%; "Clostridia"; 82%; Clostridiales; 77%; "Ruminococcaceae"; 59%; Ethanoligenens; 29%	386
VT009-D3	2	Bacteria; 98%; Firmicutes; 84%; "Clostridia"; 83%; Clostridiales; 82%; "Ruminococcaceae"; 76%; Ethanoligenens; 50%	386
VT009-H5	2	Bacteria; 100%; Firmicutes; 90%; "Clostridia"; 90%; Clostridiales; 86%; "Ruminococcaceae"; 81%; Ethanoligenens; 49%	386
VT008-D8	2	Bacteria; 100%; Firmicutes; 91%; "Clostridia"; 91%; Clostridiales; 89%; "Ruminococcaceae"; 78%; Ethanoligenens; 44%	388
VT009-C7	2	Bacteria; 100%; Firmicutes; 93%; "Clostridia"; 92%; Clostridiales; 89%; "Ruminococcaceae"; 77%; Ethanoligenens; 47%	388
VT009-F5	2	Bacteria; 100%; Firmicutes; 90%; "Clostridia"; 90%; Clostridiales; 88%; "Ruminococcaceae"; 77%; Ethanoligenens; 44%	388
VT008-F8	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 99%; Faecalibacterium; 99%	404
VT011-D2	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 100%; Faecalibacterium; 99%	404
VT009-F10	2	Bacteria; 100%; Firmicutes; 52%; "Clostridia"; 34%; Clostridiales; 34%; "Ruminococcaceae"; 24%; Fastidiosipila; 19%	426
VT013-E4	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	444
VT011-E2	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	452
VT008-H11	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	455

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VT010-F11	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	455
VT010-H11	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	455
VT012-E5	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	455
VT012-H9	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 92%	455
VT013-A5	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	455
VT013-F4	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	455
VT011-A2	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	456
VT011-F3	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	456
VT011-G1	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	456
VT012-H10	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 99%	456
VT010-B11	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT010-C11	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT010-D11	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT010-E10	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 99%	458
VT010-E12	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT010-F10	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT010-F12	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT010-G11	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT010-H12	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT011-A1	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT011-A4	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT011-A7	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT011-B1	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT011-B2	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT011-B4	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT011-B5	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT011-C10	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT011-C2	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT011-C3	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT011-C5	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT011-D5	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT011-D7	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT011-E3	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT011-E5	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT011-E7	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT011-F2	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458

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VT011-F5	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT011-F7	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT011-G3	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT011-G7	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT011-H1	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT011-H10	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT011-H3	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT011-H5	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT011-H6	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT011-H7	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT011-H8	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT012-B10	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT012-D11	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT012-F5	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458
VT009-A1	2	Bacteria; 100%; Bacteroidetes; 100%; Bacteroidetes; 100%; Bacteroidales; 100%; Rikenellaceae; 100%; Alistipes; 100%	463
VT009-F6	2	Bacteria; 100%; Bacteroidetes; 100%; Bacteroidetes; 100%; Bacteroidales; 100%; Rikenellaceae; 100%; Alistipes; 99%	463
VT009-G6	2	Bacteria; 100%; Bacteroidetes; 100%; Bacteroidetes; 100%; Bacteroidales; 100%; Rikenellaceae; 100%; Alistipes; 99%	463
VT009-D11	2	Bacteria; 100%; Firmicutes; 95%; "Clostridia"; 95%; Clostridiales; 95%; "Eubacteriaceae"; 30%; Anaerofustis; 30%	464
VT015-F10	1	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 100%	469
VT015-A11	1	Bacteria; 100%; Proteobacteria; 100%; Epsilonproteobacteria; 100%; Campylobacterales; 100%; Campylobacteraceae; 100%; Campylobacter; 100%	470
VT015-B11	1	Bacteria; 100%; Proteobacteria; 100%; Epsilonproteobacteria; 100%; Campylobacterales; 100%; Campylobacteraceae; 100%; Campylobacter; 100%	470
VT015-C11	1	Bacteria; 100%; Proteobacteria; 100%; Epsilonproteobacteria; 100%; Campylobacterales; 100%; Campylobacteraceae; 100%; Campylobacter; 100%	470
VT015-D11	1	Bacteria; 100%; Proteobacteria; 100%; Epsilonproteobacteria; 100%; Campylobacterales; 100%; Campylobacteraceae; 100%; Campylobacter; 100%	470
VT015-E11	1	Bacteria; 100%; Proteobacteria; 100%; Epsilonproteobacteria; 100%; Campylobacterales; 100%; Campylobacteraceae; 100%; Campylobacter; 100%	470
VT015-F11	1	Bacteria; 100%; Proteobacteria; 100%; Epsilonproteobacteria; 100%; Campylobacterales; 100%; Campylobacteraceae; 100%; Campylobacter; 100%	470
VT011-A10	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 97%; Shuttleworthia; 27%	470
VT009-F3	2	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 97%	471
VT013-E1	3	Bacteria; 100%; Firmicutes; 97%; "Clostridia"; 97%; Clostridiales; 92%; Clostridiaceae; 27%; Clostridiaceae 3; 21%; Thermohalobacter; 21%	475
VT011-E8	3	Bacteria; 100%; Firmicutes; 72%; "Clostridia"; 71%; Clostridiales; 70%; "Eubacteriaceae"; 14%; Pseudoramibacter; 14%	476
VT011-F8	3	Bacteria; 100%; Firmicutes; 75%; "Clostridia"; 75%; Clostridiales; 69%; "Ruminococcaceae"; 28%; Papillibacter; 10%	476
VT011-G8	3	Bacteria; 100%; Firmicutes; 72%; "Clostridia"; 71%; Clostridiales; 70%; "Eubacteriaceae"; 14%; Pseudoramibacter; 14%	476
VT013-H2	3	Bacteria; 100%; Firmicutes; 74%; "Clostridia"; 74%; Clostridiales; 71%; "Ruminococcaceae"; 22%; Papillibacter; 5%	477
VT011-D11	3	Bacteria; 100%; Firmicutes; 81%; "Clostridia"; 80%; Clostridiales; 79%; Peptococcaceae; 48%; Peptococcaceae 1; 48%; Peptococcus; 48%	478
VT011-G10	3	Bacteria; 99%; Firmicutes; 74%; "Clostridia"; 72%; Clostridiales; 66%; "Eubacteriaceae"; 35%; Pseudoramibacter; 34%	478
VT011-G11	3	Bacteria; 100%; Firmicutes; 83%; "Clostridia"; 81%; Clostridiales; 81%; Peptococcaceae; 40%; Peptococcaceae 1; 40%; Peptococcus; 40%	478
VT011-H11	3	Bacteria; 100%; Firmicutes; 84%; "Clostridia"; 83%; Clostridiales; 83%; Peptococcaceae; 44%; Peptococcaceae 1; 44%; Peptococcus; 44%	478

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VT011-G4	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	479
VT011-F11	3	Bacteria; 100%; Firmicutes; 88%; "Clostridia"; 87%; Clostridiales; 87%; Peptococcaceae; 47%; Peptococcaceae 1; 47%; Peptococcus; 47%	479
VT013-B9	1	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 57%	483
VT009-E8	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 99%; "Lachnospiraceae Incertae Sedis"; 51%	485
VT011-H9	3	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 98%; Clostridiales; 98%; "Lachnospiraceae"; 88%; Pseudobutyrivibrio; 28%	485
VT009-C11	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 99%; "Lachnospiraceae"; 99%; "Lachnospiraceae Incertae Sedis"; 40%	488
VT011-D9	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 98%; "Lachnospiraceae Incertae Sedis"; 93%	488
VT011-G9	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 98%	488
VT013-B1	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 99%; "Lachnospiraceae Incertae Sedis"; 89%	491
VT013-H7	1	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Pasteurellales; 100%; Pasteurellaceae; 100%; Gallibacterium; 100%	493
VT011-B6	3	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Pasteurellales; 100%; Pasteurellaceae; 100%; Gallibacterium; 98%	493
VT011-C6	3	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Pasteurellales; 100%; Pasteurellaceae; 100%; Gallibacterium; 99%	493
VT011-E6	3	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Pasteurellales; 100%; Pasteurellaceae; 100%; Gallibacterium; 100%	493
VT011-F6	3	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Pasteurellales; 100%; Pasteurellaceae; 100%; Gallibacterium; 100%	493
VT011-G2	3	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Pasteurellales; 100%; Pasteurellaceae; 100%; Gallibacterium; 99%	493
VT011-G6	3	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Pasteurellales; 100%; Pasteurellaceae; 100%; Gallibacterium; 99%	493
VT011-H2	3	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Pasteurellales; 100%; Pasteurellaceae; 100%; Gallibacterium; 99%	493
VT013-D7	1	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 67%	496
VT013-A10	1	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Escherichia; 42%	496
VT013-A9	1	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 71%	496
VT013-B10	1	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 96%	496
VT013-F9	1	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 90%	496
VT013-H9	1	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 100%	496
VT004-D9	2	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Escherichia; 58%	496
VT004-E9	2	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 93%	496
VT004-F9	2	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 99%	496
VT013-E9	1	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 99%	497
VT011-A9	3	Bacteria; 100%; Firmicutes; 75%; "Clostridia"; 45%; Clostridiales; 44%; "Lachnospiraceae"; 13%; Lachnobacterium; 9%	501
VT011-D12	3	Bacteria; 100%; Firmicutes; 89%; "Clostridia"; 85%; Clostridiales; 85%; "Ruminococcaceae"; 56%; Sporobacter; 19%	507
VT012-C3	3	Bacteria; 100%; Firmicutes; 88%; "Clostridia"; 80%; Clostridiales; 79%; "Lachnospiraceae"; 28%; Lachnobacterium; 10%	507
VT012-G9	3	Bacteria; 100%; Proteobacteria; 67%; Gammaproteobacteria; 66%; Pasteurellales; 66%; Pasteurellaceae; 66%; Gallibacterium; 62%	517
VT008-E10	2	Bacteria; 100%; Proteobacteria; 94%; Gammaproteobacteria; 94%; Enterobacteriales; 94%; Enterobacteriaceae; 94%; Shigella; 92%	523
VT009-B9	2	Bacteria; 100%; Firmicutes; 78%; "Clostridia"; 76%; Clostridiales; 73%; "Peptostreptococcaceae"; 6%; Sporacetigenium; 6%	524
VT009-G8	2	Bacteria; 100%; Firmicutes; 84%; "Clostridia"; 82%; Clostridiales; 81%; "Peptostreptococcaceae"; 4%; Sporacetigenium; 4%	524
VT004-H9	2	Bacteria; 100%; Firmicutes; 97%; "Clostridia"; 87%; Clostridiales; 87%; "Ruminococcaceae"; 34%; Papillibacter; 16%	525
VT009-C4	2	Bacteria; 100%; Bacteroidetes; 32%; Sphingobacteria; 31%; Sphingobacteriales; 31%; Flexibacteraceae; 31%; Leadbetterella; 29%	525

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VT009-F11	2	Bacteria; 100%; Bacteroidetes; 30%; Sphingobacteria; 27%; Sphingobacteriales; 27%; Flexibacteraceae; 27%; Leadbetterella; 21%	525
VT009-H3	2	Bacteria; 100%; Bacteroidetes; 22%; Sphingobacteria; 21%; Sphingobacteriales; 21%; Flexibacteraceae; 21%; Leadbetterella; 20%	525
VT009-H8	2	Bacteria; 100%; Firmicutes; 75%; "Clostridia"; 73%; Clostridiales; 73%; "Ruminococcaceae"; 39%; Papillibacter; 16%	525
VT012-E1	3	Bacteria; 100%; Bacteroidetes; 28%; Sphingobacteria; 26%; Sphingobacteriales; 26%; Flexibacteraceae; 26%; Leadbetterella; 26%	525
VT012-G1	3	Bacteria; 100%; Firmicutes; 85%; "Clostridia"; 82%; Clostridiales; 81%; Incertae Sedis XIII; 29%; Anaerovorax; 29%	525
VT013-E3	3	Bacteria; 100%; Firmicutes; 89%; "Clostridia"; 88%; Clostridiales; 88%; "Ruminococcaceae"; 35%; Anaerotruncus; 28%	532
VT012-H7	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 99%; Clostridiales; 99%; "Peptostreptococcaceae"; 88%; "Peptostreptococcaceae Incertae Sedis"; 62%	534
VT009-F2	2	Bacteria; 100%; Firmicutes; 97%; "Clostridia"; 96%; Clostridiales; 96%; "Ruminococcaceae"; 83%; Acetanaerobacterium; 37%	543
VT009-H4	2	Bacteria; 100%; Bacteroidetes; 31%; Sphingobacteria; 31%; Sphingobacteriales; 31%; Flexibacteraceae; 31%; Leadbetterella; 30%	545
VT012-D12	3	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 99%; Lactobacillus; 99%	568
VT012-E4	3	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 100%	568
VT012-F4	3	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 100%	568
VT012-H4	3	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 100%	568
VT013-C12	1	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 100%	569
VT013-D8	1	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 100%	569
VT013-E11	1	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 100%	569
VT013-E7	1	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 100%	569
VT015-H10	1	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 100%	569
VT012-A7	3	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 100%	569
VT012-G4	3	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 100%	569
VT013-C4	3	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 100%	569
VT013-H8	1	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 100%	570
VT013-G8	1	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 100%	572
VT013-B8	1	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 99%	596
VT013-A3	3	Bacteria; 99%; Firmicutes; 74%; "Clostridia"; 73%; Clostridiales; 69%; "Ruminococcaceae"; 45%; Papillibacter; 17%	2889
VT004-F11	2	Bacteria; 100%; Bacteroidetes; 99%; Bacteroidetes; 86%; Bacteroidales; 86%; Rikenellaceae; 82%; Alistipes; 80%	115, 95
VT004-G11	2	Bacteria; 100%; Bacteroidetes; 100%; Bacteroidetes; 95%; Bacteroidales; 95%; Rikenellaceae; 93%; Alistipes; 89%	115, 95
VT004-H12	2	Bacteria; 100%; Bacteroidetes; 99%; Bacteroidetes; 87%; Bacteroidales; 87%; Rikenellaceae; 84%; Alistipes; 82%	115, 95
VT005-C4	2	Bacteria; 100%; Bacteroidetes; 99%; Bacteroidetes; 91%; Bacteroidales; 91%; Rikenellaceae; 90%; Alistipes; 88%	115, 95
VT005-D4	2	Bacteria; 100%; Bacteroidetes; 99%; Bacteroidetes; 91%; Bacteroidales; 91%; Rikenellaceae; 90%; Alistipes; 88%	115, 95
VT005-E4	2	Bacteria; 100%; Bacteroidetes; 100%; Bacteroidetes; 96%; Bacteroidales; 96%; Rikenellaceae; 95%; Alistipes; 92%	115, 95
VT009-B6	2	Bacteria; 100%; Bacteroidetes; 100%; Bacteroidetes; 96%; Bacteroidales; 96%; Rikenellaceae; 95%; Alistipes; 92%	115, 95
VT008-A9	2	Bacteria; 100%; Firmicutes; 98%; "Clostridia"; 97%; Clostridiales; 97%; "Lachnospiraceae"; 93%; Lachnobacterium; 34%	140, 92, 82
VT005-E3	2	Bacteria; 100%; Firmicutes; 95%; "Clostridia"; 94%; Clostridiales; 93%; "Lachnospiraceae"; 77%; Lachnobacterium; 8%	144, 75
VT008-H12	2	Bacteria; 100%; Firmicutes; 95%; "Clostridia"; 94%; Clostridiales; 93%; "Lachnospiraceae"; 77%; Lachnobacterium; 8%	144, 75
VT010-C1	3	Bacteria; 98%; Firmicutes; 84%; "Clostridia"; 84%; Clostridiales; 81%; "Lachnospiraceae"; 71%; Lachnospira; 12%	144, 75

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VT008-C6	2	Bacteria; 100%; Firmicutes; 91%; "Clostridia"; 89%; Clostridiales; 87%; "Lachnospiraceae"; 68%; Catonella; 10%	144, 95
VT008-E11	2	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 99%; "Lachnospiraceae"; 99%; Syntrophococcus; 59%	149, 92, 75
VT007-D6	2	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 98%; Clostridiales; 98%; "Lachnospiraceae"; 98%; Syntrophococcus; 79%	149, 95
VT007-C1	2	Bacteria; 100%; Bacteroidetes; 100%; Bacteroidetes; 96%; Bacteroidales; 96%; Rikenellaceae; 95%; Alistipes; 92%	155, 95
VT008-D9	2	Bacteria; 100%; Bacteroidetes; 98%; Bacteroidetes; 85%; Bacteroidales; 85%; Rikenellaceae; 82%; Alistipes; 80%	155, 95
VT008-F9	2	Bacteria; 100%; Bacteroidetes; 100%; Bacteroidetes; 96%; Bacteroidales; 96%; Rikenellaceae; 95%; Alistipes; 92%	155, 95
VT008-H8	2	Bacteria; 100%; Bacteroidetes; 99%; Bacteroidetes; 91%; Bacteroidales; 91%; Rikenellaceae; 90%; Alistipes; 88%	155, 95
VT006-C10	2	Bacteria; 100%; Bacteroidetes; 99%; Bacteroidetes; 87%; Bacteroidales; 87%; Rikenellaceae; 84%; Alistipes; 82%	155, 95
VT006-F5	2	Bacteria; 100%; Bacteroidetes; 100%; Bacteroidetes; 96%; Bacteroidales; 96%; Rikenellaceae; 95%; Alistipes; 92%	155, 95
VT009-B1	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 100%; Anaerotruncus; 100%	160, 138
VT009-E4	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 78%; Dorea; 31%	172, 124, 92
VT009-H12	3	Bacteria; 100%; Firmicutes; 89%; "Clostridia"; 89%; Clostridiales; 88%; "Ruminococcaceae"; 63%; Acetanaerobacterium; 18%	177, 107
VT006-D7	2	Bacteria; 100%; Firmicutes; 54%; "Bacilli"; 45%; "Lactobacillales"; 34%; Lactobacillaceae; 33%; Lactobacillus; 33%	181, 35
VT009-C9	2	Bacteria; 100%; Firmicutes; 84%; "Clostridia"; 84%; Clostridiales; 67%; "Ruminococcaceae"; 33%; Subdoligranulum; 16%	184, 120
VT009-D10	2	Bacteria; 100%; Firmicutes; 82%; "Clostridia"; 73%; Clostridiales; 66%; "Ruminococcaceae"; 40%; Subdoligranulum; 21%	184, 120
VT009-G10	2	Bacteria; 100%; Firmicutes; 92%; "Clostridia"; 92%; Clostridiales; 86%; "Ruminococcaceae"; 72%; Subdoligranulum; 34%	189, 184, 102
VT008-E12	2	Bacteria; 100%; Firmicutes; 96%; "Clostridia"; 96%; Clostridiales; 94%; "Ruminococcaceae"; 26%; Anaerotruncus; 22%	192, 75, 15
VT007-A2	2	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 99%; "Lachnospiraceae"; 91%; Acetitomaculum; 11%	194, 75, 15
VT007-A3	2	Bacteria; 100%; Firmicutes; 95%; "Clostridia"; 95%; Clostridiales; 95%; "Lachnospiraceae"; 81%; Butyrivibrio; 9%	194, 75, 15
VT010-H7	3	Bacteria; 100%; Bacteroidetes; 100%; Bacteroidetes; 100%; Bacteroidales; 100%; Bacteroidaceae; 100%; Bacteroides; 100%	199, 83
VT006-C11	2	Bacteria; 99%; Firmicutes; 82%; "Clostridia"; 80%; Clostridiales; 76%; "Ruminococcaceae"; 24%; Sporobacter; 19%	202, 13
VT008-G11	2	Bacteria; 100%; Firmicutes; 98%; "Clostridia"; 98%; Clostridiales; 98%; "Lachnospiraceae"; 95%; "Lachnospiraceae Incertae Sedis"; 35%	205, 92
VT007-A12	2	Bacteria; 100%; Firmicutes; 98%; "Clostridia"; 98%; Clostridiales; 98%; "Lachnospiraceae"; 92%; Syntrophococcus; 39%	207, 35
VT008-C5	2	Bacteria; 100%; Firmicutes; 91%; "Clostridia"; 90%; Clostridiales; 89%; "Lachnospiraceae"; 80%; "Lachnospiraceae Incertae Sedis"; 25%	207, 35
VT012-H2	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 58%	2078, 75, 15
VT012-B4	3	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 99%	208, 37
VT009-C5	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 74%	209, 171, 92, 15
VT008-A7	2	Bacteria; 98%; Firmicutes; 95%; "Clostridia"; 94%; Clostridiales; 88%; "Lachnospiraceae"; 80%; Bryantella; 27%	211, 35
VT012-A3	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 97%; Coprococcus; 22%	218, 171, 89
VT011-B10	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 79%; Acetitomaculum; 16%	219, 190, 75
VT011-C11	3	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 99%; "Lachnospiraceae"; 84%; Lachnospira; 28%	221, 191, 75
VT013-B4	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; Bryantella; 31%	2218, 75
VT009-F1	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 39%	222, 170, 92
VT008-A10	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 54%	222, 171, 92
VT009-C2	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 88%	222, 171, 92
VT009-H2	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 62%	222, 171, 92

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VT009-B4	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 84%	222, 171, 92
VT011-E9	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 64%	222, 171, 92
VT012-A2	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 100%	222, 171, 92
VT011-B9	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 88%	222, 171, 92, 52
VT009-A5	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 99%	222, 171, 92, 72
VT009-A2	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 92%	222, 172, 95
VT012-F12	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 99%; "Lachnospiraceae Incertae Sedis"; 52%	222, 222, 92
VT008-B10	2	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 99%; "Lachnospiraceae"; 99%; Dorea; 20%	222, 75
VT008-B12	2	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 99%; "Lachnospiraceae"; 96%; "Lachnospiraceae Incertae Sedis"; 54%	222, 92
VT008-C9	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 96%; Dorea; 42%	222, 92
VT009-B2	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 50%	222, 92
VT009-B3	2	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 99%; "Lachnospiraceae"; 96%; "Lachnospiraceae Incertae Sedis"; 54%	222, 92
VT010-A7	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 95%; "Lachnospiraceae Incertae Sedis"; 70%	222, 92
VT012-E3	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 98%	2228, 75
VT012-H3	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 97%; "Lachnospiraceae"; 97%; Lachnobacterium; 20%	223, 171, 66
VT008-C10	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 52%	223, 188, 75
VT006-G4	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 95%; "Lachnospiraceae Incertae Sedis"; 62%	224, 15
VT009-F8	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 65%	224, 171, 75, 17
VT009-A4	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 55%	224, 171, 92
VT009-A8	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 89%; Dorea; 25%	224, 171, 92
VT009-B8	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 57%	224, 171, 92
VT009-C12	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 48%	224, 171, 92
VT009-E6	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 73%	224, 171, 92
VT009-H6	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 60%	224, 171, 92
VT009-H7	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; Roseburia; 39%	224, 171, 92
VT011-E10	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 72%	224, 171, 92
VT008-B11	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 51%	224, 171, 92, 52
VT013-C1	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 48%	224, 172, 92
VT009-F4	2	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 99%; "Lachnospiraceae"; 90%; "Lachnospiraceae Incertae Sedis"; 38%	224, 75
VT009-D1	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 98%; "Lachnospiraceae Incertae Sedis"; 49%	224, 76
VT009-D8	2	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 99%; "Lachnospiraceae"; 97%; "Lachnospiraceae Incertae Sedis"; 36%	224, 92
VT008-F2	2	Bacteria; 100%; Firmicutes; 98%; "Clostridia"; 98%; Clostridiales; 98%; "Lachnospiraceae"; 84%; Dorea; 35%	224, 92, 35
VT009-G11	2	Bacteria; 100%; Firmicutes; 85%; "Clostridia"; 75%; Clostridiales; 71%; "Ruminococcaceae"; 44%; Papillibacter; 11%	225, 184, 120
VT009-D5	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 97%; Dorea; 73%	226, 222, 92
VT009-E5	2	Bacteria; 100%; Firmicutes; 98%; "Clostridia"; 97%; Clostridiales; 95%; "Lachnospiraceae"; 75%; Hespellia; 32%	226, 224, 92
VT008-A4	2	Bacteria; 100%; Firmicutes; 62%; "Clostridia"; 49%; Clostridiales; 48%; Peptococcaceae; 12%; Peptococcaceae 1; 12%; Peptococcus; 12%	228, 13

SARDI ID	Trial	Taxonomical Hierarchy* (Domain, phylum, class, order, family and genus)	*Msp*I in-silico restriction fragment#
VT013-A1	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 100%; Papillibacter; 75%	243, 177, 103
VT009-B11	2	Bacteria; 100%; Bacteroidetes; 98%; Bacteroidetes; 96%; Bacteroidales; 96%; Rikenellaceae; 96%; Alistipes; 94%	263, 115, 95
VT009-H10	2	Bacteria; 100%; Bacteroidetes; 89%; Bacteroidetes; 87%; Bacteroidales; 87%; Rikenellaceae; 87%; Alistipes; 87%	263, 155, 95
VT010-A11	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 99%; "Peptostreptococcaceae Incertae Sedis"; 99%	270, 188
VT009-E2	2	Bacteria; 100%; Firmicutes; 95%; "Clostridia"; 77%; Clostridiales; 73%; "Ruminococcaceae"; 69%; Ethanoligenens; 53%	275, 176, 81
VT012-A1	3	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 99%; "Lachnospiraceae"; 71%; Coprococcus; 24%	282, 171, 17
VT013-G9	1	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 79%	283, 197
VT008-A12	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 96%; Anaerotruncus; 46%	284, 152
VT012-E2	3	Bacteria; 100%; Firmicutes; 97%; "Clostridia"; 97%; Clostridiales; 96%; "Ruminococcaceae"; 82%; "Ruminococcaceae Incertae Sedis"; 71%	285, 172, 17
VT011-H12	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 77%; Faecalibacterium; 77%	286, 188
VT013-D3	3	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 99%; "Ruminococcaceae"; 86%; Anaerotruncus; 43%	286, 189, 52
VT007-B6	2	Bacteria; 100%; Firmicutes; 56%; "Clostridia"; 56%; Clostridiales; 56%; Syntrophomonadaceae; 12%; Pelospora; 12%	287, 17
VT013-B3	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; Hespellia; 62%	287, 171
VT011-D8	3	Bacteria; 100%; Firmicutes; 90%; "Clostridia"; 90%; Clostridiales; 90%; "Ruminococcaceae"; 35%; Papillibacter; 14%	287, 185
VT011-C9	3	Bacteria; 100%; Firmicutes; 96%; "Clostridia"; 95%; Clostridiales; 95%; "Ruminococcaceae"; 88%; Fastidiosipila; 29%	287, 225, 17
VT013-A2	3	Bacteria; 100%; Firmicutes; 88%; "Clostridia"; 82%; Clostridiales; 79%; "Ruminococcaceae"; 67%; Papillibacter; 27%	288, 159, 52
VT009-E3	2	Bacteria; 100%; Firmicutes; 73%; "Clostridia"; 71%; Clostridiales; 68%; "Ruminococcaceae"; 38%; Faecalibacterium; 10%	288, 188
VT013-C2	3	Bacteria; 100%; Firmicutes; 97%; "Clostridia"; 97%; Clostridiales; 92%; "Ruminococcaceae"; 68%; Anaerotruncus; 24%	288, 189, 52
VT009-G7	2	Bacteria; 100%; Firmicutes; 79%; "Clostridia"; 76%; Clostridiales; 76%; "Ruminococcaceae"; 71%; Papillibacter; 33%	288, 190
VT013-C3	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 74%; Anaerotruncus; 24%	288, 241
VT009-F7	2	Bacteria; 100%; Firmicutes; 98%; "Clostridia"; 98%; Clostridiales; 98%; "Lachnospiraceae"; 76%; Oribacterium; 28%	289, 188
VT009-E11	2	Bacteria; 100%; Firmicutes; 75%; "Clostridia"; 74%; Clostridiales; 70%; Peptococcaceae; 25%; Peptococcaceae 1; 25%; Peptococcus; 25%	289, 189
VT013-D2	3	Bacteria; 100%; Firmicutes; 79%; "Clostridia"; 79%; Clostridiales; 76%; "Eubacteriaceae"; 27%; Pseudoramibacter; 27%	289, 190
VT013-B2	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 99%; "Ruminococcaceae"; 91%; Acetanaerobacterium; 38%	289, 191
VT013-H1	3	Bacteria; 100%; Firmicutes; 84%; "Clostridia"; 59%; Clostridiales; 58%; "Ruminococcaceae"; 30%; Fastidiosipila; 16%	289, 267
VT011-F10	3	Bacteria; 100%; Firmicutes; 87%; "Clostridia"; 87%; Clostridiales; 85%; "Ruminococcaceae"; 51%; Faecalibacterium; 19%	290, 189
VT008-C8	2	Bacteria; 100%; Firmicutes; 73%; "Clostridia"; 73%; Clostridiales; 72%; "Ruminococcaceae"; 44%; Faecalibacterium; 27%	297, 109
VT012-E12	3	Bacteria; 100%; Firmicutes; 97%; "Clostridia"; 97%; Clostridiales; 97%; "Ruminococcaceae"; 84%; "Ruminococcaceae Incertae Sedis"; 74%	298, 17
VT011-E12	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 83%; Moryella; 40%	298, 171, 17
VT013-D1	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 64%; Acetitomaculum; 26%	298, 189
VT012-B12	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 99%; Clostridiales; 99%; "Ruminococcaceae"; 74%; Anaerotruncus; 31%	298, 191
VT013-E2	3	Bacteria; 100%; Firmicutes; 97%; "Clostridia"; 97%; Clostridiales; 97%; "Ruminococcaceae"; 51%; Ethanoligenens; 19%	298, 214
VT008-F10	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 99%; "Ruminococcaceae"; 97%; "Ruminococcaceae Incertae Sedis"; 76%	298, 226, 17
VT008-G10	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 99%; "Ruminococcaceae"; 97%; "Ruminococcaceae Incertae Sedis"; 76%	298, 226, 17
VT009-A12	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Ruminococcaceae"; 97%; "Ruminococcaceae Incertae Sedis"; 72%	298, 226, 17
VT009-G2	2	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 99%; "Ruminococcaceae"; 94%; "Ruminococcaceae Incertae Sedis"; 79%	298, 226, 17

SARDI ID	Trial	Taxonomical Hierarchy* (Domain, phylum, class, order, family and genus)	*Msp*I in-silico restriction fragment#
VT007-C9	2	Bacteria; 99%; Firmicutes; 76%; "Clostridia"; 76%; Clostridiales; 76%; "Ruminococcaceae"; 41%; Faecalibacterium; 30%	298, 35
VT009-G3	2	Bacteria; 100%; Firmicutes; 96%; "Clostridia"; 96%; Clostridiales; 94%; "Lachnospiraceae"; 91%; Lachnobacterium; 20%	299, 171
VT009-D4	2	Bacteria; 100%; Firmicutes; 98%; "Clostridia"; 98%; Clostridiales; 98%; "Lachnospiraceae"; 98%; Shuttleworthia; 45%	299, 171, 52
VT012-H12	3	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 99%; "Ruminococcaceae"; 95%; Anaerotruncus; 40%	299, 226, 17
VT004-G9	2	Bacteria; 100%; Firmicutes; 98%; "Bacilli"; 51%; "Lactobacillales"; 50%; Lactobacillaceae; 13%; Paralactobacillus; 13%	299, 265
VT012-H11	3	Bacteria; 100%; Firmicutes; 99%; "Clostridia"; 99%; Clostridiales; 99%; "Ruminococcaceae"; 90%; Ruminococcus; 36%	300, 190, 52
VT012-C2	3	Bacteria; 100%; Firmicutes; 91%; "Clostridia"; 89%; Clostridiales; 87%; "Ruminococcaceae"; 67%; Anaerotruncus; 24%	302, 33
VT007-E12	2	Bacteria; 100%; Firmicutes; 95%; "Clostridia"; 95%; Clostridiales; 92%; "Ruminococcaceae"; 86%; Acetanaerobacterium; 28%	303, 35
VT008-D6	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 99%; "Ruminococcaceae"; 94%; Acetanaerobacterium; 30%	303, 35
VT007-G7	2	Bacteria; 100%; Bacteroidetes; 34%; Bacteroidetes; 30%; Bacteroidales; 30%; Bacteroidaceae; 30%; Megamonas; 30%	308 ,18
VT012-G6	3	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 74%; Bacillales; 73%; "Staphylococcaceae"; 73%; Staphylococcus; 73%	311, 155, 12
VT012-H1	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 89%	314, 171
VT013-G1	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 100%; "Lachnospiraceae Incertae Sedis"; 82%	314, 171, 52
VT011-F12	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 90%; Dorea; 52%	314, 223
VT011-G12	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 90%; Dorea; 52%	314, 223
VT009-H1	2	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Lachnospiraceae"; 98%; Hespellia; 49%	315, 171
VT012-G12	3	Bacteria; 100%; Firmicutes; 94%; "Clostridia"; 92%; Clostridiales; 92%; "Ruminococcaceae"; 73%; Papillibacter; 28%	317, 227
VT009-D12	2	Bacteria; 100%; Bacteroidetes; 100%; Bacteroidetes; 100%; Bacteroidales; 100%; Rikenellaceae; 100%; Alistipes; 100%	335, 115, 67
VT008-A11	2	Bacteria; 100%; Bacteroidetes; 100%; Bacteroidetes; 100%; Bacteroidales; 100%; Rikenellaceae; 100%; Alistipes; 100%	335, 115, 95
VT008-D10	2	Bacteria; 100%; Bacteroidetes; 100%; Bacteroidetes; 100%; Bacteroidales; 100%; Rikenellaceae; 100%; Alistipes; 100%	335, 115, 95
VT009-B5	2	Bacteria; 100%; Bacteroidetes; 100%; Bacteroidetes; 100%; Bacteroidales; 100%; Rikenellaceae; 100%; Alistipes; 100%	335, 115, 95
VT009-H11	2	Bacteria; 100%; Bacteroidetes; 100%; Bacteroidetes; 100%; Bacteroidales; 100%; Rikenellaceae; 100%; Alistipes; 100%	335, 115, 95
VT013-F1	3	Bacteria; 100%; Firmicutes; 82%; "Clostridia"; 37%; Clostridiales; 37%; Clostridiaceae; 13%; "Clostridiaceae 1"; 9%; Anaerobacter; 9%	369, 190
VT012-E8	3	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 100%	387, 189, 68
VT012-F6	3	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 100%	388, 189
VT012-H8	3	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 100%	388, 189
VT013-G4	3	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 100%	388, 189
VT012-G8	3	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 100%	388, 189
VT012-G10	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 98%	388, 68
VT012-F8	3	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 100%	389, 181
VT012-G5	3	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 100%	389, 181
VT012-H5	3	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 100%	389, 181
VT013-B5	3	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 100%	389, 181
VT010-G10	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	401, 57
VT012-A6	3	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 100%	407, 181
VT012-F1	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 99%; Clostridiales; 99%; "Ruminococcaceae"; 89%; "Ruminococcaceae Incertae Sedis"; 47%	426, 226, 17

SARDI ID	Trial	Taxonomical Hierarchy* (Domain, phylum, class, order, family and genus)	*Msp*I in-silico restriction fragment#
VT010-C12	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458, 21
VT012-E11	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458, 35
VT011-H4	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458, 52
VT010-A12	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458, 52
VT010-B12	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458, 52
VT010-E11	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458, 52
VT011-A11	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458, 52
VT011-A5	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458, 52
VT011-A8	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458, 52
VT011-B8	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458, 52
VT011-C7	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458, 52
VT011-C8	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458, 52
VT011-D1	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458, 52
VT011-D4	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458, 52
VT011-D6	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458, 52
VT011-E1	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458, 52
VT011-E4	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458, 52
VT011-F1	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458, 52
VT011-F4	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458, 52
VT011-G5	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458, 52
VT012-B11	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458, 52
VT012-B8	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458, 52
VT012-E6	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458, 52
VT012-E7	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458, 52
VT012-F10	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458, 52
VT012-G11	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458, 53
VT012-F7	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458, 68, 52
VT012-A11	3	Bacteria; 100%; Firmicutes; 100%; "Clostridia"; 100%; Clostridiales; 100%; "Peptostreptococcaceae"; 100%; "Peptostreptococcaceae Incertae Sedis"; 100%	458, 8
VT011-B11	3	Bacteria; 100%; Firmicutes; 76%; "Clostridia"; 73%; Clostridiales; 73%; "Ruminococcaceae"; 59%; Faecalibacterium; 27%	467, 52
VT011-B12	3	Bacteria; 100%; Firmicutes; 80%; "Clostridia"; 77%; Clostridiales; 75%; "Eubacteriaceae"; 15%; Anaerofustis; 15%	467, 52
VT013-F2	3	Bacteria; 100%; Firmicutes; 90%; "Clostridia"; 89%; Clostridiales; 87%; "Ruminococcaceae"; 39%; Anaerotruncus; 23%	476, 52
VT012-G7	3	Bacteria; 100%; Firmicutes; 97%; "Clostridia"; 66%; Clostridiales; 65%; "Peptostreptococcaceae"; 49%; Sporacetigenium; 47%	491, 72
VT013-E8	1	Bacteria; 100%; Proteobacteria; 100%; Gammaproteobacteria; 100%; Enterobacteriales; 100%; Enterobacteriaceae; 100%; Shigella; 89%	496, 110
VT012-A4	3	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 100%; Lactobacillus; 100%	522, 29
VT015-G10	1	Bacteria; 100%; Firmicutes; 100%; "Bacilli"; 100%; "Lactobacillales"; 100%; Lactobacillaceae; 98%; Lactobacillus; 98%	569, 45
VT013-A8	1	Bacteria; 100%; Firmicutes; 98%; "Bacilli"; 96%; "Lactobacillales"; 95%; Lactobacillaceae; 75%; Lactobacillus; 72%	588, 12

* 16S rRNA sequences were assigned to a taxonomical hierarchy using “Classifier: Naive Bayesian rRNA Classifier Version 2.0, July 2007”. A confidence threshold of greater than or equal to 80% should be applied for a reliable classification assignment.

Where a single *Msp*I restriction fragments is presented it represents a T-RF. Where multiple *Msp*I restriction fragments are presented it is not indicated which fragment may represent the T-RF of interest. Furthermore, it is possible that the sum of the fragments represents a T-RF.

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Plain English Compendium Summary

Project Title:	
Project No.:	06-25
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Objectives	-Identification of bacterial components associated with early establishment of a healthy gut microbiota, which may be used to promote beneficial life-long colonisation. -Identification of bacteria associated with improved performance traits in broilers and development of diagnostic tests for indicator bacteria associated with broiler performance.
Background	The gut microbiota may have beneficial, benign or detrimental effects on its host. Feed associated changes in the gut microbiota have previously been linked with improved performance in broilers. Understanding the dynamics of the poultry gut microbial community is necessary to develop strategies to improve feed utilisation and growth rate, avoid intestinal disease and identify better feed additives and nutrient levels that influence beneficial microbial communities. The first two weeks post-hatch have been shown to be a dynamic period for gut microbiota changes and are closely linked to gut development and enteric immunity.
Research	Broiler gut microbiota development was investigated in the first 17 days post-hatch. Influence of three in-feed antimicrobials on normal gut microbiota development was also investigated. Furthermore, linkages were established with three independent Australian broiler feeding/ performance trials. Gut microbiota was investigated in chickens across trials and common bacteria potentially linked to performance identified.
Outcomes	A complex gut microbial community was already detectable in broiler chicks 3 days post-hatch. These communities were influenced by addition of in-feed antimicrobials in different ways. Specific bacteria (Lactobacillus) were identified as contributing to the bacterial community structure and some were influenced by in-feed antimicrobials. Changes were also observed in the gut microbiota in the first 17 days post-hatch regardless of feed type. Post-hatch caecal microbial communities took longer to stabilise than ileal communities. Across the three Australian feeding trials specific bacterial groups were identified as being commonly linked to broiler performance. Three of these bacterial groups were consistently linked to improved performance, while one was linked to decreased performance. The eight performance related bacterial groups identified in this study may represent up to 22 bacterial species. Rapid diagnostic assays have been developed to identify and quantify five of these bacterial species.
Implications	Gut microbiota may be influenced as early as 3 days post-hatch which may have implications for gut development and immunity. This early, or even *in-ovo*, period needs to be investigated further for impact on life long health and performance. Diagnostic assays to potential performance related bacterial species will aid in the evaluation of feeding strategies for improving or maintaining broiler health and performance.
Publications	Torok, V.A., Ophel-Keller, K., Mikkelsen, L.L., Perez-Maldonado, R.,

	Balding, K., MacAlpine, R. and Hughes, R.J. (2010). Characterisation of gut bacteria associated with broiler performance across various Australian feeding trials. *Aust. Poult. Sci. Symp*. **21**: 195-198. Torok, V.A., Allison, G.E., Ophel-Keller, K. and Hughes, R.J. (2009). The post-hatch gut microbiota development in broiler chickens. *Aust. Poult. Sci. Sym.* **20**: 73-76. Torok, V.A., Hughes, R.J., Ophel-Keller, K., Ali, M. and MacAlpine, R. (2009). Influence of different litter materials on cecal microbiota colonization in broiler chickens. *Poult. Sci.* **88**:2474-2481. Torok VA, Ophel-Keller K, Loo M, Hughes RJ (2008) Application of methods for identifying broiler chicken gut bacterial species linked with increased energy metabolism. *Appl. Env. Microbiol*. **74**, 783-791. Torok VA, Ophel-Keller K, Hughes RJ (2008) To what extent can gut bacteria affect broiler production? *World's Poult. Sci. J.l* **64**, 125.