

The Development and Use of Molecular Tools to Monitor the Gut Microflora of Poultry

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Abstract

The microorganisms that colonise the gastrointestinal tract during the early post-hatch period form a synergistic relationship with their poultry host, releasing and providing essential nutrients as well as competitively excluding pathogenic species. Microorganisms can also directly interact with the lining of the gastrointestinal tract, which may alter the uptake of essential nutrients and the immunological status of the bird. The gastro-intestinal tract contains a complex population of bacteria, which can have both negative and positive effects on their host. However, the complexity of these interactions is not yet fully understood. This report describes the development and application of terminal restriction fragment length polymorphism (T-RFLP), a microbial profiling technique for examining the chicken intestinal microflora based on high-throughput, high resolution fingerprinting of bacterial gene regions. This tool is capable of providing a “snap-shot” of the complex bacterial population at any particular time and can be used to examine diet-induced changes in the microbial community of the chicken gut and help provide insight into how this may impact on poultry health.

Introduction

Gut microbiology and its role in animal health has become increasingly important, particularly now that the use of antibiotics in animal feeds to promote growth and/or to prevent enteric disease is constrained by legislation and consumer pressure. Gastrointestinal microorganisms have a highly significant impact on uptake and utilisation of energy (Choct *et al.*, 1996) and other nutrients (Smits *et al.*, 1997; Steinfeldt *et al.*, 1995), and on the response of poultry to anti-nutritional factors (such as non-starch polysaccharides), pre- and pro-biotic feed additives and feed enzymes (Bedford and Apajalahti, 2001). Microorganisms can also directly interact with the lining of the gastrointestinal tract (Van Leeuwen *et al.*, 2004), which may alter the physiology of the tract and immunological status of the bird (Klasing *et al.*, 1999). Current methods for analysis of intestinal flora rely on culturing, which is not only laborious but misses a large part of the uncultivable microflora. Alternatively, DNA techniques have the advantages of being rapid, relatively inexpensive and capable of monitoring gene regions of complex populations.

Currently the techniques of choice for microbial community analysis in many disciplines are denaturing or temperature gradient gel electrophoresis (DGGE/TGGE) (Muyzer, 1999). However, these techniques are not conducive to high throughput. An alternate technique for bacterial community analysis is terminal restriction fragment length polymorphism (T-RFLP) (Osborn *et al.*, 2000). Like DGGE/TGGE this technique also amplifies the 16S subunit of the bacterial ribosomal DNA present in biological samples; however, all bacterial sequences amplified are labelled with a fluorescent dye. The amplified and labelled bacterial sequences are cut with sequence specific enzymes. The resulting fragments are separated according to size and detected by fluorescence emission from the incorporated dye by a DNA sequencing machine. Results are converted to graphical profiles where peaks can represent taxonomically related groups and/or strains of bacteria. These can be easily compared between samples to identify changes in bacterial community composition. The T-RFLP technique has great potential for large scale profiling, as 96 samples can be run at a time.

Materials and Methods

Total nucleic acid was extracted from chicken gut samples by a modification of a SARDI propriety extraction method. Bacterial ribosomal DNA was amplified with universal 16S bacterial primers, one of which was 5'-labelled with 6-carboxyfluorescein. Amplicons were cut with various four base pair recognition sequence restriction enzymes and then separated on a capillary DNA sequencer (ABI 3700, Applied Biosystems). Data were analysed using GeneScan 3.7 (Applied Biosystems) to determine positions of terminal restriction fragments (TRF). Prior to statistical analysis the TRF profiles were analysed by a modified method of Dunbar *et al.*, 2001 and resulting TRF treated as operational taxonomic units (OTU). OTU were analysed using multivariate statistical models.

Results and Discussion

We have elected to use the T-RFLP technique for chicken gut microbial community analysis because of its high-throughput potential. T-RFLP, like all PCR based methods, is influenced by template and therefore DNA extraction procedure. PCR bias is also another important issue; therefore, PCR primer choice and standardized conditions are important when developing a high throughput method. To this end we have: (i) compared various DNA extraction procedures and developed an effective DNA extraction protocol suitable for chicken gut samples, which is also conducive to high throughput; (ii) compared various universal 16S ribosomal DNA PCR primers to ascertain those which perform best; (iii) tested the reproducibility of our method; and (iv) developed quality controls to be included in each T-RFLP run. The selection of restriction enzymes chosen for the T-RFLP analyses were based on those, which showed the greatest theoretical discrimination potential between available bacterial ribosomal 16S sequences in databases. These have given us good preliminary results in our T-RFLP analysis of chicken gut microbial samples.

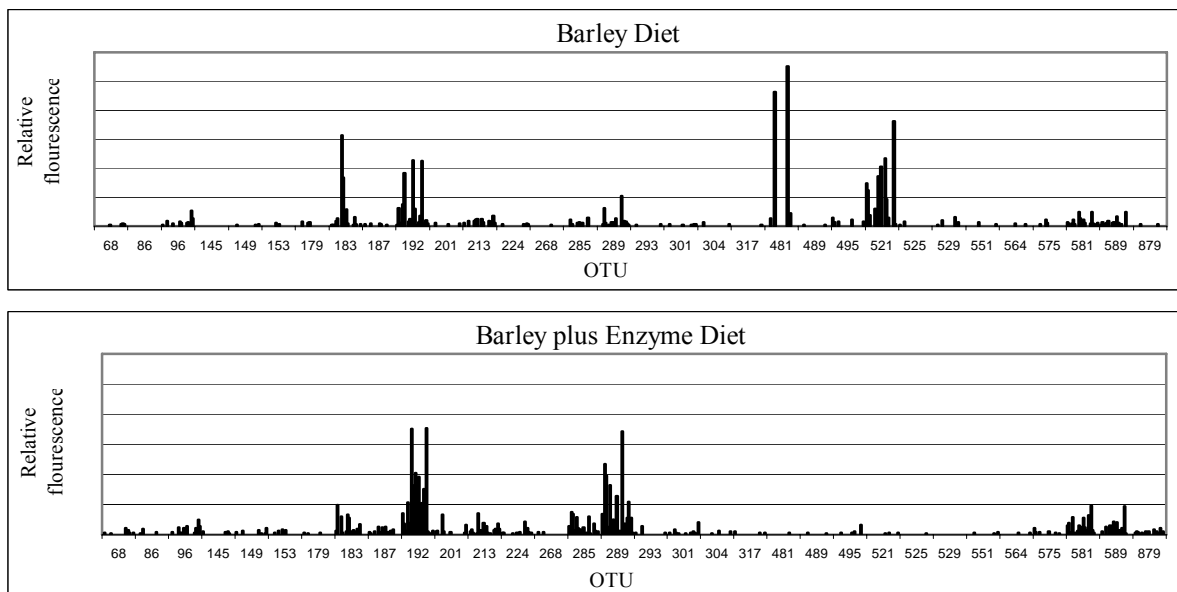


Figure 1. OTU obtained from T-RFLP analysis of the bacterial ribosomal DNA genes amplified from ileal samples from chickens fed a barley diet (n=24) or a barley plus enzyme diet (n=24).

Initial animal experiments have been done at SARDI's, Pig and Poultry Production Institute into the effects of different diets on the microbial communities of the chicken gut. This has involved raising two groups on chickens on a barley diet and a barley diet supplemented with exogenous enzyme product (Avizyme at 1100 1 kg/tonne inclusion rate) and analysing gut samples by T-RFLP. Operational taxonomic units (OTU) in Figure 1

represent taxonomically related groups and/or species of bacteria found in the chicken ileum. Peak heights are a rough representation of the proportion of different bacterial groups found in the population. Note that the most significant difference in bacterial groups between the two treatments is the presence of a taxonomically related group and/or species at an OTU of 521 in the barley diet group, which is insignificant in the barley plus enzyme diet group. Multivariate statistical analysis of OTU from the two treatments showed that there is a significance difference in the overall ileal microbial communities of chickens fed these two diets.

Conclusion

The use of our developed T-RFLP tool will allow us to define what constitutes an optimum intestinal microflora in a healthy chicken, and to utilise this technology and benchmark to conduct comparative studies of the effects of dietary manipulations on changes in the beneficial and detrimental bacterial population in the gut of chickens. This tool will contribute to an increased knowledge of the chicken gut microbiota, and hence, a better understanding in its role in chicken nutrition.

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