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Bacterial Populations are Affected by DNA Extraction and Sampling Techniques

Chengwei Li*

Department of Plant Genetics and Molecular Breeding, Zhoukou Normal University, Zhoukou, 466001, China

Abstract

The amount and quality of DNA produced, as well as its appropriateness for subsequent PCR amplifications, varied significantly depending on the DNA extraction technique used. Quantitative PCR enumeration of microbial marker loci was used to further evaluate DNA extracts from nine extraction techniques that satisfied these initial quality standards. Depending on the DNA extraction technique utilised, the same rumen samples' absolute bacteria counts varied by more than 100-fold. Using 454 Titanium pyro sequencing, the apparent compositions of the archaeal, bacterial, ciliate protozoal, and fungal communities in similar rumen samples were evaluated.

Keywords: Bacterial populations • DNA extraction • Absolute bacteria

Introduction

The relative abundances of members of the phyla Bacteroidetes and Firmicutes, for instance, varied significantly between extraction methods, indicating significant changes in the composition of the microbial community. Using one of the DNA extraction techniques, microbial populations in parallel samples taken from cows via oral stomach-tubing or through a rumen fistula, as well as in liquid and solid rumen digesta fractions, were compared. Regardless of the rumen sampling strategy employed, community representations were usually similar, although considerable variations in the abundances of specific microbial taxa, including the Clostridiales and the Methanobrevibacter ruminantium clade, were found. Prevotellaceae were most prevalent in the liquid component of the rumen sample, which had a different apparent microbial community makeup. Methods of DNA extraction that included mechanical lysis and phenol-chloroform extraction appeared to be more comparable. Comparison of data from research using other sampling methodologies, rumen sample fractions, or DNA extraction methods, however, should be done [1].

The production of meat and dairy products, wool, and leather depends heavily on ruminants like cattle, sheep, goats, deer, yak, and deer. Ruminants are kept under a wide range of farming techniques, habitats, and feeding regimes all throughout the world. The rumen is where grain is first broken down in the digestive system of ruminants. Microbes there are crucial in the digestion of dietary ingredients like fibre, resulting in short chain fatty acids that give the host energy. Thus, rumen microorganisms are crucial sources of animal nutrition and energy and are crucial to ruminant productivity. Methane is a greenhouse gas that is produced as a metabolic byproduct by rumen archaea [2].

Description

The microbial community of the rumen is very intricate. Per gramme of rumen contents, there are roughly 1011 microbial cells, of which many distinct species and genera of bacteria, archaea, fungi, ciliate protozoa, and viruses are present. Only a few numbers of these have been effectively cultivated and characterised up to this point. Molecular investigations of rumen microbial communities make it possible to identify previously undiscovered microorganisms and have become crucial techniques for identifying changes that take place within microbial communities, such as those brought on by dietary changes. Detailed microbial analyses of large-scale trials are now achievable thanks to the advent of high-throughput sequencing techniques, which also make it possible to detect minor changes in the absolute and relative numbers of microbial marker loci. For these investigations, DNA of sufficient yield and quality serves as the essential starting material. The rumen's microbial population is extremely diverse, and not all DNA extraction techniques are effective for every type of microbial population. Several studies have demonstrated that the DNA extraction technique utilised affects the representation of the microbial population in samples from various habitats, including the rumen. The method of sampling (such as oral stomach tubing and collecting through a rumen fistula) and the fractionation of the rumen sample (into liquid and solid, for example) can both affect the characteristics of the microbial population [3].

It is essential that the rumen sampling, sample fractionation, and DNA extraction methods are standardised, or at least have been shown to produce comparable results, to enable the direct comparison of rumen community structure data from studies carried out in different laboratories and around the world. The objective of this work was to thoroughly analyse various DNA extraction techniques and their effects on molecular ecological approaches' downstream analysis of rumen microbial populations. To do this, bacterial, archaeal, ciliate protozoal, fungal abundances and community compositions based on microbial marker loci were examined, as well as the quantity and quality of DNA recovered by the various approaches. Additionally, how a fistula or other method of rumen sampling affects the makeup of the micro biome [4].

The same process was used to collect samples from 16 cows' fistulae and the samples from these cows and two more from the same herd were compared to those from the comparison of rumen sampling methods (above), as well as the solid and liquid fractions. Each sample was split into a liquid fraction and a solid fraction immediately after sampling using a 0.8 mm side-by-side square hole sieve mesh (material retained by a 0.8 mm sieve mesh). The DNA was isolated from sample fractions using the PCQI method, DNA was frozen, freeze-dried, and homogenised (see Table 1 for further information), and the microbial community composition was calculated as explained below. Of all the techniques tested, PSP1, PSP2, and QIAG extracted DNA with the maximum molecular weight, but PCSA and PCBB, which also included a mechanical lysis phase, tended to produce extracts with more sheared DNA

***Address for Correspondence:** Chengwei Li, Department of Plant Genetics and Molecular Breeding, Zhoukou Normal University, Zhoukou, 466001, China; E-mail: lichengweihist@153.com*

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(data not shown). In general, the DNA retrieved from the rumen sample of the hay-fed cow was more intact than the DNA extracted from the sample of the pasture-fed sheep. Rumen contents from cows fed hay included whole particles that seemed fibrous, whereas those from sheep fed fresh pasture contained chewed-up particles that were surrounded by a viscous liquid phase appeared less fibrous. Since the DNA recovered by all methods was often larger than 1 kb, they should be beneficial for techniques that produce shorter sequences [5].

In this study, we assessed how different cell lysis and/or DNA recovery techniques affected the results of a micro biota compositional analysis of both RF and FC fractions. Thus, in order to contextualise the consequences of the DNA extraction procedure, the fraction effect was also described and discussed. Four rumen-cannulated dairy cows each fed a distinct roughage-based diet that was previously proven to cause variations in methane emission were the sources of the sample fractions. Prior to assessing the bacterial, archaeal, and fungal communities with quantitative PCR (qPCR) and 454-based pyrosequencing of barcoded 16S rRNA gene and ITS PCR amplicons, the quality and amount of the extracted genomic DNA were assessed. In order to compare various types and combinations of cell lysis mechanisms and/or DNA recovery procedures, four different DNA extraction techniques were examined. One person carried out each extraction. Eight samples-a RF sample and an FC sample-derived from four separate cows, each of which was fed a different diet-were used for each DNA extraction technique. With the exception of the sample from the cow fed the GS100 diet, for which double DNA extractions were carried out, all extractions were done just once. 0.2 g of pulverised FC or the cell pellet from 1 ml of RF was used for DNA extraction.

Conclusion

All DNA extracts were amplified using PCR, with a few exceptions, using primer pairs that targeted loci in bacteria, archaea, ciliate protozoa, and rumen fungi. Using several primer combinations, marker genes from ciliate and fungus protozoa could not be consistently amplified from the sheep sample. This was observed for all types of extraction, which suggests that the sample's low prevalence of these microbes was to blame. No one of the four microbial groups' flag genes could be amplified using DNA extracts produced by the CTAB technique. In an effort to produce PCR results from these entire DNA preparations prepared using the CTAB method, numerous adjustments to the PCR conditions were performed, but none of them were effective.

Acknowledgement

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Conflict of Interest

None.

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